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ANTIDEPRESSANT – LIKE PROPERTIES OF ACEA (ARACHIDONYL–2–CHLOROETHYLAMIDE), THE SELECTIVE AGONIST OF CB_1 RECEPTORS

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Abstract: The antidepressant effect of ACEA (arachidonyl–2–chloroethylamide), a selective agonist of CB_1 receptors, and its interaction with fluoxetine were studied in mice. ACEA (1.0 and 2.0 mg/kg *i.p.*) reduced the immobility time in the forced swimming test and attenuated the head – twitch response to L–5–HTP. The concomitant administration of ACEA (1.0 mg/kg *i.p.*) and fluoxetine (20 mg/kg *i.p.*) resulted in the strongest shortening of immobility time, significant in comparison with both ACEA and fluoxetine given alone. The obtained results indicate that ACEA may have antidepressant efficacy and shows a synergistic effect when given with fluoxetine in the forced swimming test.

Keywords: ACEA; fluoxetine; forced swimming test; L–5–HTP; cannabinoids; depression

Mammalian tissues contain two types of cannabinoid receptors, CB_1 and CB_1, both coupled to their effector systems through G (i/o) proteins. CB_1 receptors are present at high concentration in the central nervous system (cerebral cortex, limbic areas – including *hippocampus* and amygdala, basal gangalia, *cerebellum*, *thalamus* and brainstem) as well as in certain neuronal and nonneuronal peripheral tissues.

Endogenous ligands for cannabinoid receptors have also been discovered, the most important being arachidonylethanolamide (anandamide) and 2–arachidonyl glycerol (1, 2, 3).

Neuronal CB_1 cannabinoid receptors are engaged in the control of variety physiological functions as emotional states, pain, appetite, sleep, cognition, memory, learning and some pathologic conditions connected with them, such as anxiety, depression, insomnia, schizophrenia (4, 5, 6, 7, 8).

Cannabinoid receptor agonists, delta(9)–tetrahydrocannabinol (THC) and nabilone, are used clinically as antiemetics or to boost appetite (9, 10, 11). Additional therapeutic uses of cannabinoid receptor agonists may include the treatment of psychiatric diseases, particulary depression.

The aim of the present study was to investigate the antidepressant activity of ACEA (arachidonyl–2–chloroethylamide), the selective agonist of CB_1 receptors, and its potential interaction with fluoxetine. ACEA was examined in two behavioral test used to assess the antidepressive activity: the forced swimming test (FST) and head twitches induced by L–5–HTP. The effect of co–administration of ACEA and fluoxetine was examined only in the FST.

EXPERIMENTAL

Substances used

Fluoxetine (Prozac, Eli Lilly CH), ACEA (arachidonyl–2–chloroethylamide, ethanol solution, Tocris), L–5–HTP (L–5–hydroxytryptophan, Sigma). Fluoxetine and L–5–HTP were suspensed in 1% aqueous solution of Tween 80 and ACEA was diluted with distilled water. All agents were given intraperitoneally (*i.p.*) in a volume of 10 ml/kg. The controls received the same volume of 1% Tween 80.

The experiments were carried out on male BALB/c mice (18–23 g) kept under standard laboratory conditions, with free access to food and water. Each experimental group consisted of 10 animals/dose. The significance of results was evaluated by the Student's t – test.

The protocol of experiments was approved by the local Ethical Committee.

Forced swimming test (FST)

The test was performed according to Porsolt et al (12). Swim sessions were conducted by placing mice in individual cylinders (25×10 cm) filled with water (21–23°C) up to a level of 6 cm from the bottom. The animals were droped into the cylinders and left there for 6 min. Duration of immobility was assessed throughout the last 4 min. A mouse was judged to be immobile when in remained

floating, making only the movements necessary to keep its head above water. ACEA at doses of 0.5, 1.0, 2.0 mg/kg or fluoxetine at a dose of 20 mg/kg were given 1 h before the test.

The head twitch responses induced by L–5–HTP

L–5–HTP (180 mg/kg) was given 1 h after ACEA (0.5, 1.0, 2.0 mg/kg). Head twitches were recorded according to the method of Corne at al. (13). Observation of each group began immediately after L–5–HTP administration and was continued at the following time intervals: 4–6, 14–16, 24–26, 34–36, 44–46 and 54–56 min.

RESULTS

Forced swimming test

ACEA at doses of 1.0 and 2.0 mg/kg *i.p.* significantly shortened the immobility time. At a dose of 2.0 mg/kg *i.p.* its efficacy was similar to that of fluoxetine given at a dose of 20 mg/kg, which at a value of 0.5 mg/kg was inactive. The concomitant administration of ACEA, (1.0 mg/kg) and fluoxetine (20 mg/kg) resulted in the strongest shortening of the immobility time, significant in comparison with both ACEA and fluoxetine given alone (Table 1).

Table 1. The effects of ACEA, fluoxetine and concomitant administration of ACEA and fluoxetine on immobility time in the forced swimming test in mice

Compound (mg/kg *i.p.*)		Immobility time (s)	
		Mean ± SEM	%
Control		110.8 ± 7.2	100.0
Fluoxetine	20.0	60.8±7.38*	54.9
ACEA	0.5	107.6 ± 12.5	97.1
ACEA	1.0	71.6 ± 7.5*	64.6
ACEA	2.0	62.6 ± 6.5*	56.5
Fluoxetine	20.0+		
ACEA	1.0	23.8 ± 9.7*, a, b	20.6

* – $p \leq 0.05$ *vs.* control; a – $p <= 0.05$ *vs.* ACEA; b – ≤ 0.05 *vs.* fluoxetine

Table 2. The effect of ACEA on head twitches responses to L–5–HTP

Compound (mg/kg *i.p.*)		Head twitches responses	
		Mean ± SEM	%
Control		7.0 ± 0.61	100.0
ACEA	0.5	5.0 ± 0.82	71.4
ACEA	1.0	3.7 ± 0.52*	52.8
ACEA	2.0	2.5 ± 0.31*	35.7

* – $p \leq 0.05$ *vs.* control

The L–5–HTP induced head twitches

ACEA at doses of 1.0 and 2.0 mg/kg significantly and dose–dependently inhibited the number of head twitch episodes induced by L–5–HTP, the lowest dose of ACEA (0.5 mg/kg) was without effect (Table 2).

DISCUSSION AND CONCLUSIONS

The present study has shown that ACEA (the CB_1 receptor agonist) induced a significant anti–immobility effect in the FST. At a dose of 2.0 mg/kg its action was similar to that produced by clinically established antidepressant drug – fluoxetine (20 mg/kg).

The FST is a behavioral test in rodents that predicts the clinical efficacy of antidepressant treatments. Many of the major classes of antidepressants reduced immobility time in the FST, including tricyclic antidepressants, monoamino oxidase inhibitors, atypical antidepressants and also a 5–HT uptake inhibitor – fluoxetine.

The neurochemical base of a positive result of fluoxetine in this test in thought to be connected with serotoninergic activation *via* $5\text{–}HT_{2C}$ receptors (14, 15, 16). In our experiment this action of fluoxetine was potentiated by ACEA.

In 5–HTP test, ACEA at doses of 1.0 and 2.0 mg/kg reduced the frequency of head–twitch episodes induced by L–5–HTP. This result corroborates those obtained by Janoyan at al. (17) who have observed that THC and other cannabinoids block the ability of DOI, a selective $5\text{–}HT_{2A}/5\text{–}HT_{2C}$ agonist, to produce the head–twitch response. In addition, it was found that SR 141716A, the selective CB_1 receptor antagonist/inverse agonist induces the head–twitch response in mice (17, 18).

The head twitch reaction induced by 5–HTP is mediated by $5\text{–}HT_{2A}$ (19) receptors and it is blocked by antidepressant drugs which possesses antiserotoninergic properties (20).

The obtained results indicate that CB_1 receptors could play a role in the mechanism of depression and in the antidepressant activity. This finding is in agreement with the study of Martin at al. (21) who observed a higher sensitivity to exhibit depressive–like responses in CB_1 knockout mice in the chronic unpredictable mild stress.

These data also suggest an interaction between cannabinoid and serotoninergic systems, which may play a role in the action of antidepressant drugs.

Additionally, our study demonstrated that the concomitant administration of CB_1 agonist and

fluoxetine may evoke a more effective antidepressive activity than the treatment with typical antidepressant given alone. This finding may be of particular importance in the case of drug–resistant patients.

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