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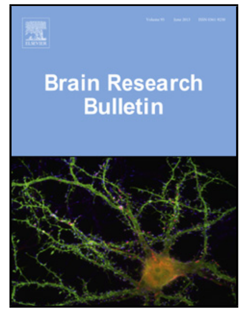

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Ligands of the CB_2 cannabinoid receptors augment activity of the conventional antidepressant drugs in the behavioural tests in mice

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Highlights

- JWH133 increases activity of conventional antidepressant drugs
- AM630 potentiates activity of conventional antidepressant drugs
- Interplay between CB_2 receptor ligands and antidepressants is pharmacodynamic in nature

Abstract

Although a lot of information can be found on the specific dual role of the endocannabinoid system in the emotional-related responses, little is known whether stimulation or inhibition of the CB receptors may affect the activity of the frequently prescribed antidepressant drugs. Our interests have been particularly focused on the potential influence of the CB_2 receptors, as the ones whose central effects are relatively poorly documented when compared to the central effects of the CB_1 receptors. Therefore, we evaluated the potential interaction between the CB_2 receptor ligands (i.e., JWH133 – CB_2 receptor agonist and AM630 – CB_2 receptor inverse agonist) and several common antidepressant drugs that influence the monoaminergic system (i.e., imipramine, escitalopram, reboxetine). In order to assess the antidepressant-like effects we used two widely recognized behavioural tests, the mouse forced swim test (FST) and the tail suspension test (TST). Brain concentrations of the tested antidepressants were evaluated by the HPLC method. Intraperitoneal co-administration of *per se* ineffective doses of JWH133 (0.25 mg/kg) or AM630 (0.25 mg/kg) with imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) significantly shortened the immobility time of mice in the FST and the TST, whereas it did not disturb their spontaneous locomotor activity. Furthermore, the brain levels of antidepressants were not changed. Summarizing, the results of the present study revealed that both activation and inhibition of the CB_2 receptor function have a potential to strengthen the antidepressant activity of drugs targeting the monoaminergic system. Most probably, the described interaction has a pharmacodynamic background.

Keywords: JWH133, AM630, imipramine, reboxetine, escitalopram, antidepressant activity

1. Introduction

The endocannabinoid system has attracted clinicians attention for decades as an endogenous homeostatic system associated with a large number of neurotransmitters and their pathways, and implicated in numerous physiological functions, including an inflammatory process, pain, or emotions. The endocannabinoid system consists of the endogenous arachidonate-based lipids referred to as "endocannabinoids" (such as anandamide and 2-arachidonoylglycerol), enzymes responsible for their synthesis (N-acylphosphatidylethanolamine-phospholipase D and diacylglycerol lipase) and degradation (fatty acid amide hydrolase and monoacylglycerol lipase), and the endocannabinoid CB receptors (for review see [1,2]). Thus far, two types of typical CB receptors, encoded by CNR1 and CNR2 genes, have been described. Both CB_1 and

CB_2 receptors bind endogenous and exogenous cannabinoids with high affinity. Due to specific mechanism of action of CB receptors, on the one hand the endocannabinoid system can help to restore the brain balance after exposure to different stressors, but on the other hand, it is involved in the pathogenesis of certain mental disturbances, like anxiety, stress, aggressive behavior, or depression. Until recently, only CB_1 receptors were thought to be implicated in the development of mental diseases as the ones that are expressed both in the periphery and the brain. CB_2 receptors were mainly considered as the peripheral receptors. It was believed that they were distributed almost exclusively within the immune cells and thus, that they have only the immunomodulatory role (i.e., regulation of cytokines or migration of immune cells) (for review see [1-3]). However, several research team have found out that CB_2 receptors are also localized in the rodent [4] and human central nervous system [5-7]. At first, their presence in the brain was linked to existence of pathological conditions, like Alzheimer's disease [8], multiple sclerosis, amyotrophic lateral sclerosis [9], or tumours [10], but then, they were also identified under physiological conditions [5]. CB_2 receptors are localized in the spinal nucleus, olfactory nucleus, thalamus, hippocampus, amygdala, cerebral cortex, and cerebellum [11,12]. They are mainly distributed post-synaptically, but they can also be found in the presynaptic areas. Functional effects of CB_2 receptors that prove their presence in the brain have also been described in literature. It has been revealed that CB_2 receptors are involved in the inflammatory responses that accompany the neurodegenerative processes [8], in stress-induced neuroinflammation [13], schizophrenia, alcohol preference in mice, alcoholism in humans [14], drug abuse, motor function, and emotionality [4]. Onaivi et al. [4] reported a reduced expression of CB_2 receptors in the striatum and midbrain of mice that developed alcohol preference.

Involvement of CB_2 receptors in the development of depression has also been suggested. Deletion of CB_2 receptors in mice with C57BL/6J congenic background induced depression-like behavior in the TST [15], whereas genetically-induced lowered CB_2 receptor function (in the *Cnr2* heterozygote knock-out mice) was associated with an increased susceptibility to depression when exposed to immune or emotional stressors (i.e., poly I:C or chronic mild stress, respectively) [16]. Reduction of CB_2 receptors in the hippocampus was observed in mice that had been subjected to the chronic unpredictable mild stress [17]. In turn, García-Gutiérrez et al. [17] found out that the genetically-induced overexpression of the CB_2 receptors exerted an antidepressant-like effects in the TST and the novelty-suppressed feeding test in mice with swiss ICR congenic background. Moreover, these animals presented a higher level of the brain-derived neurotrophic factor (BDNF) in the hippocampus and they were resistant to stressful depressogenic-like situations in the chronic unpredictable mild stress model. Both stimulation of CB_2 receptors by their selective agonists (GW405833, JWH133, or β-caryophyllene) [18-20] as well as inhibition by their selective inverse agonist (AM630) [17,20] produced an antidepressant-like activity in the FST and/or the TST in naïve albino Swiss [20], C57BL/6 [19], ICR Swiss [17] mice, or in rats that underwent chronic constriction injury [18]. Additionally, treatment with CB_2 ligands inhibited the development of the depressive-like behaviour in mice [17] and rats [21] subjected to the chronic unpredictable stress. Ishiguro et al. [14] suspected association between genetic variants of the CB_2 gene and depression in Japanese people.

Although a lot of information can be found on the specific dual role of the endocannabinoid system in the emotional-related responses, little is known whether stimulation or inhibition of CB receptors may affect the activity of the frequently prescribed antidepressant drugs. This subject seems to be quite important, in the view of the fact that more than 322 million people suffer from depression and that the efficacy of available drugs is not satisfactory. Hence, new (even controversial) treatment options are necessary. Our interests have been particularly focused on the potential influence of the CB_2 receptors, as the ones whose central effects are relatively poorly documented when compared to the central effects of the CB_1 receptors. For this purpose, we evaluated the potential interaction between CB_2 receptor ligands (i.e., JWH133 – CB_2 receptor agonist and AM630 – CB_2 receptor inverse agonist) and three common antidepressant drugs that influence the monoaminergic system (i.e., imipramine – a tricyclic antidepressant, escitalopram – a selective serotonin reuptake inhibitor, and reboxetine – a selective noradrenaline reuptake inhibitor). According to the literature data [22], JWH133 has a very high affinity towards CB_2 receptors (i.e., Ki = 3.4 ± 1 nM) and exhibits 200-fold selectivity for them over CB_1 receptors. AM630 also binds selectively to CB_2 receptors, with a CB_2/CB_1 affinity ratio of 165 and Ki = 31.2 nM at the CB_2 receptor [23]. Being an inverse agonist of CB_2 receptors, AM630 has both affinity towards CB_2 receptors and negative intrinsic activity. It binds to the constitutively activated receptors and decreases their activity below the basal level [24]. Thus, AM630 acts oppositely to JWH133. In order to assess the antidepressant-like effects we used two widely recognized behavioural tests, the mouse FST and the TST.

2. Materials and methods

2.1. Animals

The experiments were carried out on male Albino Swiss mice (25-30 g). Animals were maintained in standard cages (8 mice/cage) placed in environmentally controlled rooms (humidity ca. 45-55%, temperature of 22-23 ºC, 12 h light/dark cycle). They were given water and food *ad libitum*. All performed procedures were planned in accordance with binding law related to the experimental studies on animal models, and they were approved by the Local Ethics Committee.

2.2. Drug administration

JWH133 ((6aR,10aR)-3-(1,1-Dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran, 0.25 mg/kg, Tocris) and AM630 (6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone, 0.25 mg/kg, Tocris) were suspended in Tween 80 solution (1%). Imipramine hydrochloride (15 mg/kg, Sigma-Aldrich), escitalopram oxalate (2 mg/kg, Sigma-Aldrich), and reboxetine mesylate (2.5 mg/kg, Abcam Biochemicals) were dissolved in physiological saline. The tested suspensions/solutions were administered intraperitoneally (*ip*): imipramine, escitalopram, and reboxetine were given 60 min before behavioural tests, whereas JWH133 and AM630 were given 30 min before behavioural tests. The pretreatment schedules and doses were selected on the basis of the literature data [25] and were confirmed in the preliminary tests carried out in our lab. The control mice received *ip* injections of vehicles.

2.3. Forced swim test (FST)

The FST was performed according to method that we used before [26]. Each mouse was placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C and it was left there for 6 min. The immobility time of animals was recorded between the 2^{nd} and the 6^{th} min of the test. A given mouse was judged immobile when it stopped struggling and remained floating motionless in the water. Movements necessary to keep animal's head above the water level were acceptable.

2.4.Tail suspension test (TST)

The TST was performed according to the method that we used before [27]. Each mouse was suspended by the tail (2 cm from the end of the tail) using adhesive tape and it was left hanging for 6 min. Though the immobility time in the TST can be measured for the whole duration of the test, we recorded the activity of animals for the last 4 min. Similarly to the situation observed in the FST, animals at the beginning of the TST usually show an increased escape-oriented struggling, which lasts ca. 1-2 min [28]. In order to avoid potential confounding of the results, the first 2 min of the test was treated as a habituation period. A given mouse was judged immobile when it stopped moving its body and limbs. Only movements necessary to breathe were acceptable.

2.5. Spontaneous locomotor activity

Spontaneous locomotor activity was measured automatically, as was described before [26]. We used an activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, USA) that contains plexiglas cages with lids (43 × 43 × 32 cm) equipped with a set of four infrared emitters and four detectors monitoring mice movements. Each animal was placed individually in the cage. A distance travelled by a given animal was recorded between the 2nd and the 6th min of the test. This time interval corresponded with the one analyzed in the TST and the FST.

2.6. Determination of the antidepressant levels in brain homogenates

New cohorts of animals were used for the biochemical studies. The tested mice were decapitated 60 min after injection of the antidepressant drug (with or without JWH133 or AM630). Their brains were dissected and frozen. Imipramine, desipramine (i.e., an active metabolite of imipramine), escitalopram, and reboxetine brain levels were determined with use of the high-performance liquid chromatography (HPLC) method, as was described before [26]. The assays were reproducible with low intra- and interday variation (coefficient of variation <10%). The extraction efficiencies of the analyzed compounds and the internal standard ranged from 66% to 95%. Concentrations of antidepressants were expressed for the wet brain tissue in ng/g.

2.7. Statistical analysis

t-test or two-way analysis of variance (ANOVA) followed by Bonferroni's *post-hoc* test were applied, depending on the experimental design. The outcomes were given as the means ± standard error of the mean (SEM). When $p < 0.05$, differences between the compared groups were treated as significant.

3. Results

3.1. Effects of a concurrent administration of JWH133 and the tested antidepressants in the FST

A single administration of JWH133 (0.25 mg/kg) or a given antidepressant drug (i.e., imipramine – 15 mg/kg, escitalopram – 2 mg/kg, or reboxetine – 2.5 mg/kg) did not significantly influence the immobility time of animals in the FST. When the tested substances were injected in respective combinations, the treated mice were swimming for a longer time than their control counterparts (Fig. 1A). Two-way ANOVA presented: (1) a significant JWH133-imipramine interaction [$F(1,28)=8.57$; $p=0.0067$] with a significant effect of JWH133 [$F(1,28)=10.24$; $p=0.0034$] and a significant effect of imipramine [$F(1,28)=23.14$; $p<0.0001$], (2) a significant JWH133-escitalopram interaction [$F(1,26)=4.95$; $p=0.0351$] with a significant effect of JWH133 [$F(1,26)=8.85$; $p=0.0063$] and a significant effect of escitalopram [$F(1,26)=22.22$; $p<0.0001$], and (3) a significant JWH133-reboxetine interaction [$F(1,28)=10.87$; $p=0.0027$] with a significant effect of JWH133 [$F(1,28)=12.89$; $p=0.0012$] and a significant effect of reboxetine [$F(1,28)=27.14$; $p<0.0001$].

3.2. Effects of a concurrent administration of AM630 and the tested antidepressants in the FST

Concurrent administration of the sub-effective doses of AM630 (0.25 mg/kg) and the antidepressant drug (i.e., imipramine – 15 mg/kg, escitalopram – 2 mg/kg, or reboxetine – 2.5 mg/kg) increased activity of the tested mice in the FST, which was presented in Fig. 2A. Two-way ANOVA demonstrated: (1) a significant AM630-imipramine interaction [$F(1,26)=4.34$; $p=0.0472$] with a significant effect of AM630 [$F(1,26)=11.98$; $p=0.0019$] and a significant effect of imipramine [$F(1,26)=15.29$; $p=0.0006$], (2) a significant AM630-escitalopram interaction [$F(1,24)=6.63$; $p=0.0166$] with a significant effect of AM630 [$F(1,24)=13.94$; $p=0.0010$] and a significant effect of escitalopram [$F(1,24)=12.42$; $p=0.0017$], and (3) a significant AM630-reboxetine interaction [$F(1,28)=7.79$; $p=0.0094$] with a significant effect of AM630 [$F(1,28)=10.14$; $p=0.0035$] and a significant effect of reboxetine [$F(1,28)=14.32$; $p=0.0007$].

3.3. Effects of a concurrent administration of JWH133 and the tested antidepressants in the TST

Mice treated with a single injection of JWH133 (0.25 mg/kg) or a given antidepressant drug (i.e., imipramine – 15 mg/kg, escitalopram – 2 mg/kg, or reboxetine – 2.5 mg/kg) stayed immobile for the same duration of time as the animals from the control group. However, when the tested substances were injected in respective combinations, the antidepressant-like effect was detected in the TST (Fig. 1B). The following significant drug-drug interactions were revealed by two-way ANOVA analysis: (1) a significant JWH133-imipramine interaction [$F(1,26)=4.73$; $p=0.0389$] with a significant effect of JWH133 [$F(1,26)=8.17$; $p=0.0083$] and a significant effect of imipramine [$F(1,26)=16.48$; $p=0.0004$], (2) a significant JWH133-escitalopram interaction [$F(1,28)=8.73$; $p=0.0063$] with a significant effect of JWH133 [$F(1,28)=12.82$; $p=0.0013$] and a significant effect of escitalopram [$F(1,26)=12.42$; $p=0.0015$], and (3) a significant JWH133-reboxetine interaction [$F(1,28)=5.56$; $p=0.0255$] with a significant effect of JWH133 [$F(1,28)=8.13$; $p=0.0081$] and a significant effect of reboxetine [$F(1,28)=16.25$; $p=0.0004$].

3.4. Effects of a concurrent administration of AM630 and the tested antidepressants in the TST

After a single administration of AM630 (0.25 mg/kg) or a given antidepressant drug (i.e., imipramine – 15 mg/kg, escitalopram – 2 mg/kg, or reboxetine – 2.5 mg/kg), behavior of the treated mice in the TST was similar to the one observed for animals from the control group. By contrast, co-administration of AM630 with a given antidepressant drug significantly reduced the immobility time of animals (Fig. 2B). Two-way ANOVA demonstrated: (1) a significant AM630-imipramine interaction [$F(1,28)=9.18$; $p=0.0052$] with a significant effect of AM630 [$F(1,28)=10.38$; $p=0.0019$] and a significant effect of imipramine [$F(1,28)=10.65$; $p=0.0029$], (2) a significant AM630-escitalopram interaction [$F(1,24)=4.60$; $p=0.0424$] with a significant effect of AM630 [$F(1,24)=6.28$; $p=0.0194$] and a significant effect of escitalopram [$F(1,24)=18.63$; $p=0.0002$], and (3) a significant AM630-reboxetine interaction [$F(1,28)=9.64$; $p=0.0043$] with a significant effect of AM630 [$F(1,28)=11.26$; $p=0.0023$] and a significant effect of reboxetine [$F(1,28)=25.20$; $p<0.0001$].

3.5. Effects of a concurrent administration of JWH133 or AM630 and the tested antidepressants on the spontaneous locomotor activity of mice

As presented in Table 1, neither a single administration of the tested CB_2 receptor ligands or antidepressants nor their respective combinations influenced the spontaneous locomotor activity of animals.

3.6. Pharmacokinetic studies

Outcomes of the pharmacokinetic studies demonstrated that none of the tested cannabinoid CR_2 receptor ligands (i.e., JWH133 – 0.25 mg/kg or AM630 – 0.25 mg/kg) elevated the brain levels of imipramine, desipramine, escitalopram, and reboxetine (Table 2). *t*-test revealed the following results: (1) $t(14) = 1.276$, $p = 0.2226$ for the JWH133-imipramine (15 mg/kg) combination, (2) $t(14) = 0.6404$, $p = 0.5323$ for the JWH133-desipramine combination, (3) $t(18) = 0.7445$, $p = 0.4689$ for the JWH133-escitalopram (2 mg/kg) combination, (4) $t(14) = 0.1032$, $p = 0.9193$ for the JWH133-reboxetine (2.5 mg/kg) combination, (5) $t(14) = 0.3359$, $p = 0.7419$ for the AM630-imipramine (15 mg/kg) combination, (6) $t(14) = 0.1095$, $p = 0.9144$ for the AM630-desipramine (an active metabolite of imipramine) combination, (7) $t(14) = 0.8318$, $p = 0.4195$ for the AM630-escitalopram (2 mg/kg) combination, and (8) $t(14) = 0.005376$, $p = 0.9958$ for the AM630-reboxetine (2.5 mg/kg) combination.

4. Discussion

In the course of intensive studies over the pathomechanism of depression, the endocannabinoid system has emerged as an important item in the development and/or treatment of this disease. Medical and scientific bases provide discrepant information on the cannabinoids effects in mood disorders. Both the pro- and antidepressant activity have been recorded after cannabis consumption, and these effects use to be attributed exclusively to CB_1 receptors. However, quite recently it has been proved that CB_2 receptors can also be found in different brain areas, including those responsible for emotions, like the prefrontal cortex, hippocampus, or amygdala [4,11,17,29]. Thus, CB_2 receptors seem to be also involved in mood changes in humans, though their impact is still relatively poorly documented. Several authors suggest that CB_2 receptors could be an interesting novel target for the treatment of depressive disorders, including the post-stroke depression [17,21].

The antidepressant potential of JWH133 (0.5-1 mg/kg) – the CB_2 receptor agonist applied in our study, was observed by Kruk-Słomka et al. [20] in the FST in Swiss mice. The authors demonstrated that the JWH133 treatment exerted the U-shaped anti-immobility pattern, with ineffectiveness of the lowest (0.25 mg/kg) and the highest (2 mg/kg) tested doses. As for the AM630 – the CB_2 receptor inverse agonist, results from the behavioural studies are controversial. In the above-mentioned experiments carried out by the team of Kruk-Słomka [20], only its lowest tested dose (i.e., 0.5 mg/kg) was effective in the FST, whereas the concentrations of 1-3 mg/kg did not increase the swimming time of animals. Quite contrary were the outcomes reported by García-Gutiérrez et al. [17] who demonstrated that an acute administration of 1-3 mg/kg of AM630 produced an antidepressant-like activity in the FST, but only in the wild type of swiss ICR mice; these doses did not change the behaviour of animals with experimentally-induced overexpression of the CB_2 receptors. In fact, the authors revealed that after an acute and chronic administration of AM630 different effects in the biochemical and behavioural tests measuring the depression level in rodents may be recorded, depending on the applied experimental conditions. A 4-week administration of this CB_2 receptor ligand in the stressed wild-type ICR Swiss mice prevented the development of depression-like behaviour measured by the TST and by the sucrose intake test, whereas such a treatment had no impact on the behaviour of the non-stressed wild-type animals. Furthermore, the AM630 therapy *per se* did not influence the expression of the CB_2 receptor, BDNF gene, or the BDNF protein level in the hippocampus, but this CB_2 receptor ligand significantly reduced the BDNF loss and reversed the reduction in CB_2 receptor levels induced by the unpredictable chronic mild stress [17].

To our knowledge, the present study is the first one that assessed the influence of CB_2 receptor ligands on the activity of conventional antidepressant drugs that affect the monoaminergic system. We examined the impact of co-administration of CB_2 receptor agonist (JWH133) or CB_2 receptor antagonist (AM630) and imipramine (a tricyclic antidepressant), escitalopram (a selective serotonin reuptake inhibitor), or reboxetine (a selective inhibitor of noradrenalin reuptake) on the depression-related behaviour of mice in the FST and the TST, i.e. two most widely used animal tests evaluating the antidepressant-like activity. We demonstrated that JWH133 and AM630 when administered in *per se* inactive dose of 0.25 mg/kg significantly intensified the antidepressant effects of the applied drugs (also given at the sub-effective doses: 15 mg/kg of imipramine, 2 mg/kg of escitalopram, and 2.5 mg/kg of reboxetine), suggesting an addition or synergism of action. Mice that received single injections of respective combinations (imipramine-, escitalopram-, or reboxetine-CB_2 receptor ligand) were more mobile in the FST and the TST than animals from the control groups. According to the literature data [30,31] this observation can be treated as an indicator of the antidepressant behaviour. Similarly to our observations, both JWH133 and AM630 when given in a non-effective dose of 2 mg/kg significantly potentiated the antidepressant activity of an antagonist of the muscarinic acetylcholine receptors, i.e. scopolamine, in the FST in Swiss mice [20]. Though there are reports that CB_2 receptor agonists and antagonists may alter spontaneous locomotor activity of animals in a strain- and gender-dependent manner [4], we did not notice any differences in locomotion of mice from the tested and control groups. Similarly, in experiments by Kruk-Słomka et al. [20] no stimulant/inhibitory action of JWH133 (0.25-2 mg/kg) or AM630 (0.5-3 mg/kg) was noticed. Consequently, it is improbable that the antidepressant-like effects observed in the behavioural tests in the present study were false positive.

Generally, the molecular mechanism of the antidepressant-like activity of CB receptor ligands seems to be very complicated and complex. It has not been fully understood yet. Strangely enough, both CB_1 and CB_2 receptor agonists and antagonists may exert the antidepressant-like activity in rodents as well as they can induce the pro-depressive behaviour in animals. Moreover, it was found out that administration of the inactive dose of both CB_2 receptor antagonist (AM630, 2 mg/kg) and CB_1 antagonist (AM251, 0.25 mg/kg) is able to abolish the antidepressant-like effects of the active doses of CB_2 receptor agonist (JWH133, 0.5 and 1 mg/kg) in the FST in naïve Swiss mice. Similarly, in the same strain of rodents, administration of the non-effective dose of both CB_2 receptor antagonist (AM630, 2 mg/kg) and CB_1 antagonist (AM251, 0.25 mg/kg) is able to reverse the antidepressant-like effects of the active doses of CB_1 receptor agonist (oleamide, 10 and 20 mg/kg) [20]. In fact, Onaivi et al. [4] noted that in different neuronal populations CB_1 and CB_2 receptors work to a certain point independently, but to a certain point also cooperatively in regulation of diverse physiological activities affected by cannabinoids. Viewing the above-mentioned, it should not be surprising that both JWH133 and AM630, i.e., the compounds that should act oppositely, potentiated the activity of the tested antidepressants in our study. Most probably, elevation of the serotonin and/or noradrenaline levels leading to potentiation of the serotonergic and/or noradrenergic neurotransmissions was responsible for the observed effects. Both the tested antidepressant drugs as well as cannabinoids are believed to modulate the monoaminergic system [32]. Franklin et al. [33] demonstrated that CB_2 receptors are implicated in the cannabinoid-induced upregulation of the serotonergic $5\text{-}HT_{2A}$ receptors in the brain. This effect is most probably mediated by internalization of CB_2 receptors and stimulation of the ERK1/2 signalling pathway. Disturbances in the $5\text{-}HT_{2A}$ receptor-dependent signalling are involved in the pathomechanism of different neuropsychiatric disorders, like schizophrenia, anxiety, or depression [34,35]. It is also possible that CB_2 receptors modulate the depression-related responses *via* alteration in immune system. Their increased expression in the brain was observed after an intra-striatal administration of lipopolysaccharide in a rat model of Parkinson's disease and it corresponded with an activation of the microglia [36]. In experiments by Zoppi and colleagues [13], activation of CB_2 receptors by experimentally-induced overexpression or administration of an CB_2 receptor agonist in ICR Swiss mice exerted the anti-inflammatory or neuroprotective effects *via* control of glutamate uptake, whereas CB_2 receptors knock-out potentiated stress-induced neuroinflammatory responses. A specific role of neuro-immune interactions in the development of psychiatric disorders has been examined for decades and a range of diverse studies have demonstrated a clear link between chronic immune responses and occurrence of depression [37]. Stimulation of CB_2 receptors in the brain is also known to reduce expression of inducible nitric oxide synthase, production of nitric oxide, generation of reactive oxygen/nitrogen species, and thus, to attenuate the oxidative stress [38]. Additionally, neither JWH133 nor AM630 are exclusively specific for CB_2 receptors, but they are only CB_2 receptor-selective. They also exhibit partial affinity for CB_1 receptors [39,40]. Therefore, pathways connected with the CB_1 receptors may also contributed to the effects detected in our experiments.

Since the pharmacokinetic assays showed that neither JWH133 nor AM630 enhanced the brain levels of the tested antidepressant drugs (i.e., imipramine with its active metabolite – desipramine, escitalopram, or reboxetine), it should be assumed that the potentiation of the antidepressant effect observed in our study took place in the pharmacodynamic phase instead of in the pharmacokinetic one. However, based on the experiments of Smaga et al. [41], it is highly unlikely that an acute administration of the antidepressant drugs applied in our study might have changed the expression of endocannabinoid receptors (either CB_1 or CB_2) in the mouse brain. Such effects couldn't be ruled out after prolonged (14-day) treatment. Reduced levels of CB_1 and/or CB_2 receptors were detected in the dorsal striatum, nucleus accumbens, and/or cerebellum of male Wistar rats exposed to imipramine (15 mg/kg), whereas increased levels of CB_1 and/or CB_2 receptors in the hippocampus and/or prefrontal cortex with decreased levels of CB_1 receptors in dorsal striatum were recorded after escitalopram treatment (10 mg/kg) [41]. In the view of the above, further studies over the molecular mechanism of the interactions demonstrated in our experiments are needed.

5. Conclusions

The results of the present study revealed that both activation and inhibition of the CB_2 receptor function have a potential to strengthen the antidepressant activity of drugs targeting the monoaminergic system. Most probably, the described interaction has a pharmacodynamic background. Though our findings should be treated as the preliminary ones, we assume that the modulation of the endocannabinoid neurotransmission *via* CB_2 ligands could be a promising strategy as an adjuvant treatment in patients with depression. Such an approach could have additional benefits, since the CB_2 selective ligands are thought to be devoid of central nervous system side effects that may limit the clinical use of other substances with antidepressant potential [19].

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Figure legends

Fig. 1. Effect of a combined intraperitoneal administration of JWH133 and antidepressant drugs in (A) the FST and (B) the TST in mice.
Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas JWH133 (0.25 mg/kg) was given 30 min before the experiment. The values represent mean + SEM (n=7-8 mice per group). ^^p<0.01, ^^^p<0.01 versus respective antidepressant drug; ***p<0.001 versus JWH133 (two-way ANOVA followed by Bonferroni's *post-hoc* test).

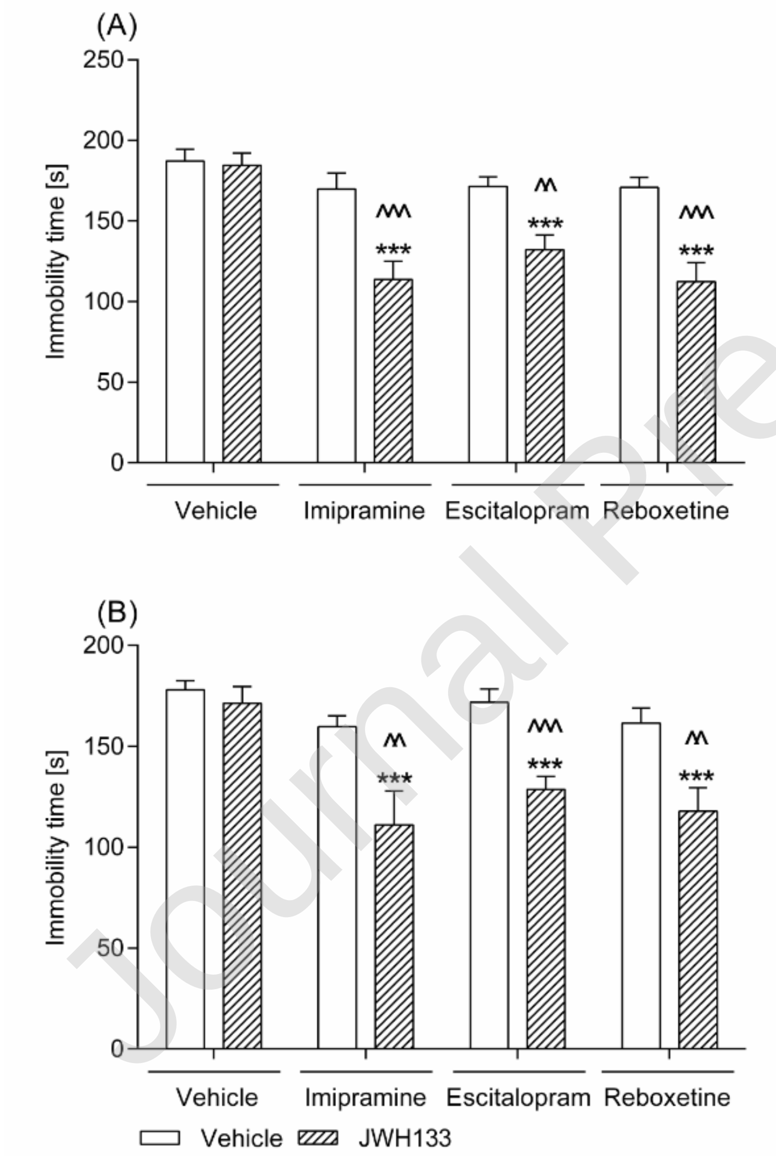

Fig. 2. Effect of a combined intraperitoneal administration of AM630 and antidepressant drugs in (A) the FST and (B) the TST in mice.

Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas AM630 (0.25 mg/kg) was given 30 min before the experiment. The values represent mean + SEM (n=7-8 mice per group). ^^p<0.01, ^^^p<0.01 versus respective antidepressant drug; ***p<0.001 versus AM630 (two-way ANOVA followed by Bonferroni's *post-hoc* test).

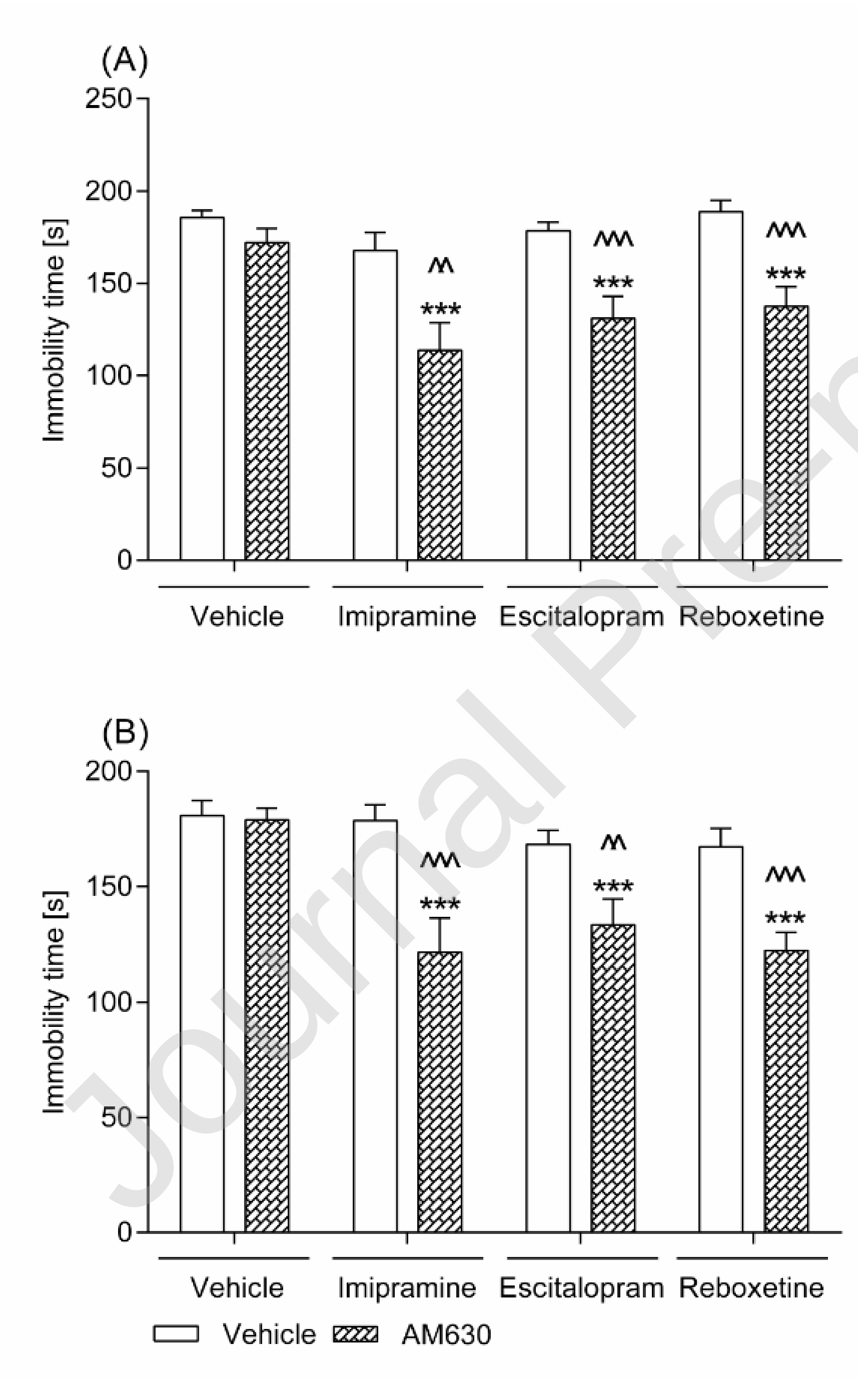

Table 1. Effect of a combined intraperitoneal administration of (A, B) JWH133 or (C, D) AM630 and antidepressant drugs on the spontaneous locomotor activity of mice

	Treatment	Travelled distance (cm)
(A)	vehicle + vehicle	474.4 ± 49.66
	JWH133 + vehicle	400.6 ± 51.81
	imipramine + vehicle	365.0 ± 46.23
	imipramine + JWH133	517.4 ± 87.69
	reboxetine + vehicle	310.0 ± 55.38
	reboxetine + JWH133	418.4 ± 55.94
(B)	vehicle + vehicle	613.0 ± 48.68
	JWH133 + vehicle	597.6 ± 50.41
	escitalopram + vehicle	777.4 ± 78.87
	escitalopram + JWH133	784.87 ± 47.74
(C)	vehicle + vehicle	526.0 ± 41.97
	AM630 + vehicle	572.5 ± 48.02
	imipramine + vehicle	559.6 ± 66.81
	imipramine + AM630	562.7 ± 38.49
	escitalopram + vehicle	667.9 ± 63.89
	escitalopram + AM630	818.3 ± 41.97
(D)	vehicle + vehicle	518.5 ± 68.85
	AM630 + vehicle	388.4 ± 53.41
	reboxetine + vehicle	366.4 ± 54.92
	reboxetine + AM630	432.5 ± 17.95

Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas JWH133 (0.25 mg/kg) and AM630 (0.25 mg/kg) were given 30 min before the experiment. The values represent mean + SEM (n=7-8 mice per group). Two-way ANOVA was used for statistical analysis.

Table 2. Effect of JWH133 and AM630 on the brain levels of antidepressants in mice

Treatment	Drug/metabolite level in the brain (ng/g)	Treatment	Drug/metabolite level in the brain (ng/g)
imipramine + vehicle imipramine + JWH133	*imipramine level* 22407 ± 3021 18011 ± 1654 *desipramine level* 2118 ± 484.4 1720 ± 386.9	imipramine + vehicle imipramine + AM630	*imipramine level* 5358 ± 1098 5989 ± 1525 *desipramine level* 441.3 ± 74.48 430.0 ± 70.79
escitalopram + vehicle escitalopram + JWH133	*escitalopram level* 507.1 ± 46.28 430.5 ± 91.96	escitalopram + vehicle escitalopram + AM630	*escitalopram level* 88.89 ± 18.40 70.84 ± 11.51
reboxetine + vehicle reboxetine + JWH133	*reboxetine level* 51.49 ± 6.134 52.48 ± 7.348	reboxetine + vehicle reboxetine + AM630	*reboxetine level* 54.95 ± 6.298 54.90 ± 7.158

Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas JWH133 (5 mg/kg) and AM630 (0.25 mg/kg) were given 30 min before decapitation. The values represent mean ± SEM (n=6-8 mice per group). *t*-test was used for statistical analysis.