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PII: S0361-9230(19)30298-9
DOI: https://doi.org/10.1016/j.brainresbull.2019.05.021
Reference: BRB 9698

To appear in: Brain Research Bulletin

Received date: 16 April 2019
Revised date: 28 May 2019
Accepted date: 29 May 2019


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Influence of the CB₁ cannabinoid receptors on the activity of the monoaminergic system in the behavioural tests in mice

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Highlights
- Oleamide potentiates activity of conventional antidepressant drugs
- AM251 enhances activity of conventional antidepressant drugs
- Interplay between CB₁ receptor ligands and antidepressants is pharmacodynamic in nature

Abstract
Antidepressants that target the monoaminergic system are prescribed most frequently in the psychiatric practice. However, not all patients benefit from their use. It is generally known that co-administration of agents aiming distinct targets may increase the therapeutic effect and
at the same time permit dose reduction. A number of studies have suggested a CB₁ receptor-mediated interplay between the endocannabinoid system and the monoaminergic signalling in the brain. Therefore, we wanted to determine whether the CB₁ receptor ligands (oleamide and AM251) affect the activity of the common antidepressant drugs that influence the monoaminergic system. In order to determine the antidepressant-like activity, the forced swim test and the tail suspension test in mice were used. Additionally, brain concentrations of the tested antidepressants were evaluated by the HPLC method. Concurrent intraperitoneal administration of per se inactive doses of oleamide (5 mg/kg) or AM251 (0.25 mg/kg) and imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) reduced the immobility time of animals in the forced swim test and the tail suspension test. The observed effect was not associated with hyperlocomotion of animals. Summarizing, the outcomes of the present study demonstrated that modulation (i.e., activation or inhibition) of the CB₁ receptor function potentiates the antidepressant activity of common drugs that influence the monoaminergic (serotonergic and noradrenergic) system. This effect is most probably predominantly pharmacodynamic in nature instead of pharmacokinetic.

Keywords: oleamide; AM251; serotonergic system; forced swim test; tail suspension test; mice

1. Introduction

_Cannabis sativa_ has been known for centuries as a folk medicinal plant effective in pain, nausea, seizures, insomnia, migraine, cough, or loss of appetite. After identification of the main biologically active substance of the cannabis, i.e., Δ⁹-tetrahydrocannabinol (Δ⁹-THC), it turned out that this component binds specifically to certain receptors present in the human brain. In consequence, these receptors have been called the endocannabinoid type 1 (CB₁) receptors, and their endogenous ligands (such as anandamide, 2-arachidonylglycerol, and others) – the endocannabinoids (Maurya and Velmurugan, 2018).

Since the medicinal world is still searching for novel, more safe and clinically effective options in the treatment of depressive disorders, the scientists and clinicians have been testing diverse promising strategies, including use of agents specifically targeting the endocannabinoid system. In fact, dysregulation of the endogenous cannabinoid system as one of the factors contributing to the development of mental diseases (including depression) was proven years ago in the pre-clinical and clinical studies. Abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis with impaired glucocorticoid secretion, altered hippocampal
release of the brain-derived neurotrophic factor (BDNF), and prominent changes in the brain expression of genes involved in the pathomechanism of depression were detected in mice experimentally deprived of CB1 receptors (Aso et al., 2011). These biochemical alterations were accompanied by depression-like behavior (Aso et al., 2008). Furthermore, animals with fatty acid amide hydrolase (FAAH) knock-out showed antidepressive behaviour in the modified tail suspension test (Naidu et al., 2007). On the other hand, rodents with experimentally induced depression presented several abnormalities in the endocannabinoid system (Vinod et al., 2012; Dubreucq et al., 2012; Eisenstein et al., 2010; Hill et al., 2008). Similarly, diverse anomalies in the endocannabinoid system were detected in suicide victims (Vinod et al., 2005) and depressed patients (Hill et al., 2005).

CB1 receptors have been found in brain structures responsible for emotions, such as the prefrontal cortex, hippocampus, amygdala, cerebellum, dorsal striatum, and nucleus accumbens (Micale et al., 2013). It was demonstrated that CB1 receptors are mainly localized in the presynaptic part of axons that release different neurotransmitters, like glutamate, gamma-aminobutyric acid (GABA), acetylcholine, noradrenaline, serotonin (Mendiguren et al., 2018). Therefore, CB1 receptors can affect neurotransmissions other than the endocannabinoid one. A number of studies have suggested a CB1 receptor-mediated interplay between the endocannabinoid system and the monoaminergic signalling in the brain (Bambico et al., 2008; Häring et al., 2007). There are evidences that cannabinoids can modulate the firing of monoaminergic neurons and influence the release of monoamines, including serotonin and norepinephrine (Sagredo et al. 2006, Mendiguren and Pineda, 2006). Furthermore, it has been observed that cannabinoids influence the activity of (α1, α2A, β) adrenoreceptors and serotonin (5HT1A, 5HT1B, 5HT7) receptors (Seyrek et al., 2010; Sagredo et al. 2006; Romero et al., 2013; Mendiguren et al., 2018), and endocannabinoid receptors are involved in the regulation of the activity of serotonin and noradrenaline transporters (SERT and NAT) (Kenny et al., 1999; Mendiguren et al., 2018). Fisar (2010) reported that high doses of cannabinoids inhibit the activity of monoamine oxidase (MAO). On the other hand, it turned out that activation of the postsynaptic sertonergic receptors 5-HT2A participates in the synthesis of endocannabinoids. It seems that the endocannabinoid-serotonergic interaction is not limited to the mood and the brain. Quite recently, the team led by Nasehi (2017) has shown the participation of the 5-HT4 receptors in learning deficits induced by the activation of CB1 receptors. Seyrek with colleagues (2010) have confirmed the involvement of the descending spinal 5-HT2A and 5-HT7 receptors in the CB1 receptor-related antinociception of systemic cannabinoids, whereas Romero et al. (2013) demonstrated implication of the
adrenergic mechanism in the peripheral antinociception elicited by CB₁ and CB₂ receptor agonists.

Regarding the important role of the endocannabinoid system with CB₁ receptors in the pathophysiology of depression as well as their interaction with the monoaminergic (serotonergic and noradrenergic) neurotransmission, we wanted to determine whether the CB₁ receptor ligands (oleamide and AM251) affect the activity of the common antidepressant drugs (imipramine, escitalopram, reboxetine) that influence the monoaminergic system. Antidepressants that target the monoaminergic system are prescribed most frequently in the psychiatric practice. However, not all patients benefit from their use. Amongst several problems that clinicians could face when recommending this type of drugs, insufficient efficacy, bothersome side effects, and delayed onset of the antidepressant activity should be particularly mentioned. It is generally known that co-administration of agents aiming distinct targets may increase the therapeutic effect and at the same time permit dose reduction. Therefore we suspect that concomitant use of substances that influence the serotonergic/noradrenergic and endocannabinoid systems may be an interesting treatment strategy. Such a polytherapy could be particularly attractive given the fact that CB₁ receptor ligands are characterized by a fast onset of their biological activity.

2. Materials and methods

2.1. Animals

All experiments were performed on adult (8-10 weeks old) male Albino Swiss mice weighing between 25 and 30 g. Animals were kept in environmentally controlled rooms (with 12 h light/dark cycle, temperature of 22-23°C, and relative humidity about 45-55%) in standard cages (425 mm × 265 mm × 150 mm), 8 mice/cage. The bedding was corncob granules and it was changed once a week. Water and food was given ad libitum. Each experimental group consisted of 8-10 subjects. The total number of animals used in the experiments was 452. All procedures were approved by the Local Ethics Committee. They were planned and performed in accordance with binding European and Polish law related to the experimental studies on animal models.

2.2. Drug administration

Oleamide (cis-9,10-octadecenoamide, 5 mg/kg, Tocris) and AM251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, 0.25 mg/kg, Tocris) were suspended in a 1% solution of Tween 80, whereas 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 (0.9 % NaCl). All solutions/suspensions were prepared immediately prior to the experiments and were administered intraperitoneally (ip): the antidepressant drugs were given 60 min before testing and the CB₁ receptor ligands were injected 30 min before testing. The doses and pretreatment schedules were selected on the basis of our previous experiments (Poleszak et al., 2016a; Wośko et al., 2018). Animals from the control groups received either i.p. injection of saline (vehicle-treated group, AM251-treated group and oleamide-treated group) or i.p. injection of a 1% solution of Tween 80 (vehicle-treated and antidepressant drug-treated groups). The volume of all administered liquid dosage forms was 10 ml/kg.

2.3. Forced swim test (FST)
The FST was carried out according to the method by Porsolt et al. (1977). Each mouse was placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C. The animals were left in the cylinder for 6 min. The immobility time of mice was recorded during the last 4 min of the 6-min testing period. An animal was judged immobile when it ceased struggling and remained floating motionless in the water, only movements necessary to keep its head above the water level were acceptable.

2.4. Tail suspension test (TST)
The TST was carried out according to the method by Steru et al. (1985). Each mouse was suspended for 6 min by the tail (2 cm from the end of the tail) using adhesive tape. The immobility time of mice was recorded during the last 4 min of the 6-min testing period. An animal was judged immobile when it ceased moving its limbs and body, only movements necessary to breathe were acceptable.

2.5. Spontaneous locomotor activity
Measurement of the spontaneous locomotor activity was carried out automatically in an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, USA), according to the procedure we had described previously (Poleszak et al., 2016b). Plexiglas cages with lids (43 × 43 × 32 cm) were equipped with a set of four infrared emitters and four detectors monitoring mice movements. Each animal was placed individually. A distance travelled by a tested mouse was measured during the last 4 min of the 6-min testing period, which corresponded with the time interval analyzed in the FST and the TST.
2.6. Determination of the antidepressant levels in brain homogenates
Animals were decapitated 60 min after administration of the antidepressant drug (with or without the respective CB1 receptor ligand) and their brains were appropriately dissected from the skull and frozen. Brain levels of imipramine, desipramine (i.e., an active metabolite of imipramine), escitalopram, and reboxetine were determined by a high-performance liquid chromatography (HPLC) method, as had been previously described (Poleszak et al., 2016a). 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 wet brain tissue in ng/g.

2.7. Statistical analysis
For statistical analysis the t-test or two-way analysis of variance (ANOVA) followed by Bonferroni’s post-hoc test were used, depending on the experiment. The obtained results were presented as the means ± standard error of the mean (SEM). The differences between the tested groups were considered as significant when p < 0.05 (i.e., *p < 0.05, **p < 0.01, ***p < 0.001).

3. Results
3.1. Effects of a co-administration of oleamide and antidepressant drugs in the FST
As presented in Fig. 1A, a single administration of oleamide (5 mg/kg), imipramine (15 mg/kg), escitalopram (2 mg/kg) or reboxetine (2.5 mg/kg) did not significantly change mice behavior in the FST. However, when these substances were given in respective combinations, the tested animals were less immobile than those from the control groups. Consequently, two-way ANOVA demonstrated: (1) a significant oleamide-imipramine interaction [F(1,36)=5.83; p=0.0209] with a significant effect of oleamide [F(1,36)=9.38; p=0.0041] and a significant effect of imipramine [F(1,36)=14.95; p=0.0004], (2) a significant oleamide-escitalopram interaction [F(1,36)=9.58; p=0.0038] with a significant effect of oleamide [F(1,36)=13.79; p=0.0007] and a significant effect of escitalopram [F(1,36)=18.30; p=0.0001], (3) a significant oleamide-reboxetine interaction [F(1,36)=6.71; p=0.0137] with a significant effect of oleamide [F(1,36)=10.37; p=0.0027] and a significant effect of reboxetine [F(1,36)=18.21; p=0.0001].
3.2. Effects of a co-administration of AM251 and antidepressant drugs in the FST

Co-administration of per se inactive doses of AM251 (0.25 mg/kg) and imipramine (15 mg/kg), escitalopram (2 mg/kg) or reboxetine (2.5 mg/kg) significantly prolonged mobility time of animals subjected to the FST (Fig. 2A). Two-way ANOVA showed the following results: (1) a significant interaction between AM251 and imipramine \([F(1,36)=27.28; p<0.0001]\) with significant effects of both AM251 \([F(1,36)=15.39; p=0.0004]\) and imipramine \([F(1,36)=48.26; p<0.0001]\), (2) a significant interaction between AM251 and escitalopram \([F(1,24)=5.27; p=0.0308]\) with significant effects of both AM251 \([F(1,24)=8.95; p=0.0063]\) and escitalopram \([F(1,24)=16.78; p=0.0004]\), (3) a significant interaction between AM251 and reboxetine \([F(1,36)=17.30; p=0.0002]\) with significant effects of both AM251 \([F(1,36)=9.57; p=0.0038]\) and reboxetine \([F(1,36)=19.86; p<0.0001]\).

3.3. Effects of a co-administration of oleamide and antidepressant drugs in the TST

Neither a single injection of oleamide (5 mg/kg) nor antidepressant drug (imipramine, 15 mg/kg; escitalopram, 2 mg/kg; reboxetine, 2.5 mg/kg) considerably affected animals behavior in the TST. By contrast, when the tested agents were given in combinations, the anti-immobility effects were recorded, which was illustrated in Fig.1B. Statistical analysis detected significant drug interactions: (1) oleamide-imipramine interaction \([F(1,32)=6.28; p=0.0175]\) with a significant effect of imipramine \([F(1,32)=25.76; p<0.0001]\) but not significant effect of oleamide \([F(1,32)=3.16; p=0.0852]\), (2) oleamide-escitalopram interaction \([F(1,36)=6.10; p=0.0184]\) with a significant effect of both oleamide \([F(1,36)=7.52; p=0.0095]\) and escitalopram \([F(1,36)=12.94; p=0.0010]\), (3) oleamide-reboxetine interaction \([F(1,34)=4.30; p=0.0457]\) with a significant effect of reboxetine \([F(1,34)=21.06; p<0.0001]\) and a not significant effect of reboxetine \([F(1,34)=4.30; p<0.0457]\).

3.4. Effects of a co-administration of AM251 and antidepressant drugs in the TST

Animals treated with a single injection of AM251 (0.25 mg/kg), imipramine (15 mg/kg), escitalopram (2 mg/kg), or reboxetine (2.5 mg/kg) stayed immobile for the same duration of time as mice given the saline. However, a concurrent administration of AM251 with an antidepressant induced a significant increase in animals’ mobility in the TST (Fig. 2B). Calculations carried out with two-way ANOVA demonstrated: (1) a significant AM251-imipramine interaction \([F(1,18)=6.66; p=0.0154]\) with a significant effect of AM251 \([F(1,28)=5.64; p=0.0247]\) and a significant effect of imipramine \([F(1,28)=19.31; p=0.0001]\), (2) a significant AM251-escitalopram interaction \([F(1,28)=39.17; p<0.0001]\) with a
significant effect of AM251 [F(1,28)=18.07; p=0.0002] and a significant effect of escitalopram [F(1,28)=48.44; p<0.0001], (3) a significant AM251-reboxetine interaction [F(1,28)=9.70; p=0.0042] with a significant effect of AM251 [F(1,28)=8.62; p=0.0066] and a significant effect of reboxetine [F(1,28)=13.94; p=0.0009].

3.5. Effects of a co-administration of oleamide or AM251 and the antidepressant drugs on the spontaneous locomotor activity of mice
Neither respective combinations of the tested agents nor their singular injections affected spontaneous locomotor activity of animals (Table 1).

3.6. Pharmacokinetic studies
The pharmacokinetic studies revealed that neither oleamide (5 mg/kg) nor AM251 (0.25 mg/kg) increased the brain levels of the tested antidepressants when given concurrently, and that the differences between the compared groups were not statistically significant. The only exception was the oleamide-imipramine (15 mg/kg) combination. Oleamide significantly enhanced the brain level of imipramine (t-test: t(18) = 2.126, p = 0.0476), but at the same time it did not influence the brain concentration of desipramine, i.e. an active metabolite of imipramine (t-test: t(18) = 0.1396, p = 0.8906). As for other co-treatments, the t-test showed the following results: (1) t(18) = 0.3996, p = 0.6941 for oleamide-escitalopram (2 mg/kg) combination, (2) t(18) = 0.2842, p = 0.7795 for oleamide-reboxetine (2.5 mg/kg) combination, (3) t(18) = 0.6072, p = 0.5513 for AM251-imipramine (15 mg/kg) combination and t(18) = 0.2938, p = 0.7723 for AM251-desipramine combination, (4) t(12) = 1.096, p = 0.2948 for AM251-escitalopram (2 mg/kg) combination, and (5) t(18) = 0.8180, p = 0.4240 for AM251-reboxetine (2.5 mg/kg) combination. The obtained results were presented in Table 2.

4. Discussion
Studies over the biological activity of cannabis revealed that the endocannabinoid signalling is quite complicated, and its final effects are affected not only by CB receptors but also by other (downstream) targets, including the serotonergic and noradrenergic systems. Literature data indicate that, at least in rodents, both direct and indirect mechanisms are involved in the regulation of the monoaminergic neurotransmission by cannabinoids. Yoshida et al. (2013) and Oropeza et al. (2006) revealed that CB1 receptors are frequently localized on the serotonergic and noradrenergic axons, respectively, and that the endocannabinoids modify...
local monoaminergic neurotransmission in different parts of the brain. Furthermore, McLaughlin and colleagues (2009) observed that activation of the HPA axis by cannabinoids at a high dose is partially mediated by a non-direct stimulation of the monoaminergic neurotransmission, with involvement of the α1- and β-adrenergic as well as the 5-HT1 and 5-HT2A/2C serotoninergic receptors. Coupling of the monoaminergic system with the endocannabinoid neurotransmission was also confirmed by the studies of Hill et al. (2006, 2008), who found out that a 3-week exposure to desipramine, fluoxetine, or tranylcypromine led to an increased density of CB1 receptors and/or altered levels of anandamide and 2-arachidonylloglycerol in the rat brain.

Viewing the above-mentioned, the outcomes of the present experiments are quite understandable. We found out that both oleamide and AM251 injected at per se ineffective doses (5 mg/kg and 0.25 mg/kg) potentiated the antidepressant activity of drugs from different pharmacological classes that influence the serotonergic/noradrenergic system, i.e. (1) imipramine (15 mg/kg) – a representative of the tricyclic antidepressants, (2) escitalopram (2 mg/kg) – belonging to the selective serotonin reuptake inhibitors, and (3) reboxetine (2.5 mg/kg) – a selective inhibitor of noradrenaline reuptake. The tested animals were significantly more active in the FST and the TST than their control counterparts, and this effect was a little bit better pronounced in the FST than in the TST. Though the theoretical basis of these tests is common, most probably, the observed behavioural responses are due to different biological substrates. Primarily, the obtained results may be partially influenced by the fact that the baseline immobility of Albino Swiss mice is a little bit lower in the TST versus the FST. Additionally, the applied behavioural tests have different sensitivity to the immobility-reducing effects of substances that affect the serotonergic neurotransmission. Therefore, diverse patterns of the dose–response curves may be obtained (Cryan et al., 2005). The anti-immobility action of oleamide and AM251 has been reported before in pre-clinical studies. These CB1 receptor ligands at the doses of 10 and 0.5–1 mg/kg, respectively, exerted an antidepressant-like activity similar to the one observed for fluoxetine and desipramine (Akanmu et al., 2007; Kruk-Słomka et al., 2015; Takahashi et al., 2008; Ostadhadi et al., 2016). Moreover, we demonstrated before that both oleamide and AM251 are able to augment the antidepressant potential of agents that inhibit the glutamatergic neurotransmission (Wośko et al., 2018). Interestingly enough, oleamide and AM251 act oppositely: oleamide is an agonist of CB1 receptors, whereas AM251 belongs to the selective CB1 receptor antagonists/inverse agonists. Bidirectional effects in relation to mood produced by cannabinoids are observed both in laboratory models and in humans. This dual activity is
explained in several different ways: (1) by the functional pools of receptors that are involved in manifestation of the depressogenic and anti-depressant effects (Patel and Hillard, 2009), (2) by the testing conditions (i.e., environmental factors, drug dose, animal strain) that could be responsible for induction of either antidepressant-like or depressive-like changes in the endogenous cannabinoid system (Hill and Gorzalka, 2005), or (3) by the existence of other subtypes of the endocannabinoid receptors that have not been described yet (Ostadhadi et al., 2016). It should be noted, that the results obtained in the behavioural tests of the present study, were not falsified by altered spontaneous locomotion of the animals. Given the stimulating effects of cannabis experienced by certain users it is important that neither single administration of the tested agents nor injection of their respective combinations induced hyperlocomotion in mice.

Our results are partially in line with the experiments by Takahashi et al. (2008), who demonstrated that combination of AM251 or SR141716A (another CB1 receptor antagonist) with the selective serotonin reuptake inhibitors (citalopram or sertraline) given at the sub-threshold doses produced an additive effect in both the FST and the TST. Surprisingly, antidepressant activity of reboxetine and a selective dopamine reuptake inhibitor – nomifensine, was not potentiated by the applied cannabinoids. Differences in experiment conditions and mice strains were most probably responsible for the discrepancy between our outcomes and the results of Takahashi’s team in relation to co-administration of AM251 and reboxetine. However, since cannabinoids represent a specific class of substances with bidirectional biological activity, we admit that other, unknown factors could have contributed to the observed effects. Particularly, as in our studies and in work by Takahashi et al. (2008) AM251 potentiated the antidepressant activity of the selective serotonin reuptake inhibitors, whereas in experiments by Umathe et al. (2011) pretreatment with this CB1 receptor antagonists/inverse agonists at a dose of 1 µg/mouse (given intracerebroventricularly) blocked the behavioural effects of fluoxetine in the Porsolt test. In addition, the intracerebroventricular administration of AM251 (0.05- 5 µg/mouse) did not produce the anti-immobility effect in the FST by itself.

We suppose that the additive anti-immobility effects noticed in our study were mainly due to an increase in serotonin and/or noradrenaline levels and due to potentiation of the serotonergic and/or noradrenergic signaling. The applied antidepressant drugs modulate the monoaminergic neurotransmission and several authors have shown that the monoaminergic system is implicated in the mood related behaviours induced by cannabinoids (Mendiguren et al., 2018). Though neither oleamide nor AM251 directly interact with the adrenergic
receptors, both of them interplay with the serotonergic ones. Oleamide modulates serotonergic signalling via enhancement of the 5HT$_{1A}$, 5HT$_{2A}$ and 5HT$_{2C}$ receptors response as well as via inhibition of the 5HT$_{7}$ receptors (Boger et al., 1998). The 5HT$_{1A}$ receptors are partially responsible for the behavioural effects observed after AM251 administration (McLaughlin et al., 2017). Following Linge et al. (2016), it is highly possible that just as in the case of cannabidiol, the 5HT$_{1A}$ receptors are involved in the rapid-acting antidepressant-like activity of oleamide and AM251. We also expect that other, non-serotonergic/adrenergic signalings may contribute to the final outcomes of the behavioural tests. Based on the available data, oleamide also affects the dopaminergic, glutaminergic, and GABA-ergic neurotransmission (Akanmu et al., 2007), whereas AM251 modulates the opioid pathways (Ostadhadi et al., 2016). All of them are directly or indirectly involved in the pathomechanism of depression.

Results of the pharmacokinetic assays carried out in the present study confirmed that the interactions between the CB$_1$ receptor ligands and the applied antidepressant drugs are probably due to modifications at the cellular level. Neither oleamide nor AM251 induced significant alterations in the brain concentrations of escitalopram or reboxetine. Though administration of oleamide increased the brain levels of imipramine, this effect was not accompanied by a significant change in desipramine (an active metabolite of imipramine) concentration. Therefore, we assume that the augmentation of antidepressant activity of the tested drugs was pharmacodynamic in nature, however in the case of imipramine-oleamide interaction, it could have taken place partially in the pharmacokinetic phase.

The positive interaction discovered in the present experiments seems to be important from the clinical point of view. Since CB$_1$ receptors are localized in specific brain areas, exposure to cannabis is associated with amnesia, dizziness, motor impairment, or mood changes (Moreira et al., 2009). On the other hand, common antidepressants targeting the monoaminergic system may induce nausea, fatigue, drowsiness, insomnia, irritability, agitation, anxiety, sexual problems, and others. Concomitant administration of substances affecting the monoaminergic and endocannabinoid systems enables reduction of dose of the used agents and thus, may improve tolerance of the prescribed treatment. Furthermore, it takes at least 4-8 weeks to observe a full spectrum of positive effects of the antidepressant treatment with typical drugs, whereas addition of cannabinoids, which are famous for generating very fast behavioural responses, may accelerate the biological processes.

5. Conclusion
Summarizing, the outcomes of the present study demonstrated that modulation (i.e., activation or inhibition) of the CB₁ receptor function potentiates the antidepressant activity of common drugs that influence the monoaminergic (serotonergic and noradrenergic) system. This effect is most probably pharmacodynamic in nature instead of pharmacokinetic. Though the obtained results require further pre-clinical and clinical confirmation, substances targeting the endocannabinoid system seem to be an attractive option as an adjuvant therapy to the standard one in depressive patients.

6. Funding
This study was supported by Funds for Statutory Activity of Medical University of Lublin, Poland.
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Figure legends

Fig. 1. Effect of a combined intraperitoneal administration of oleamide 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 oleamide (5 mg/kg) was given 30 min before the experiment. The values represent mean + SEM (n=number of mice per group). ***p<0.001 versus vehicle-treated group; ^p<0.05, ^^p<0.01, ^^^p<0.01 versus respective antidepressant drug; ###p<0.001 versus oleamide (two-way ANOVA followed by Bonferroni’s post-hoc test).

Fig. 2. Effect of a combined intraperitoneal administration of AM251 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 AM251 (0.25 mg/kg) was given 30 min before the experiment. The values represent mean + SEM (n=number of mice per group). ***p<0.001 versus vehicle-treated group; ^p<0.01, ^^p<0.01 versus respective antidepressant drug; ###p<0.001 versus AM251 (two-way ANOVA followed by Bonferroni’s post-hoc test).
Figure A: Immobility time [s] for different treatments: Vehicle, Imipramine, Escitalopram, and Reboxetine. The data is presented for two different groups: n=10 and n=10. Significant differences are indicated by symbols (###, **, *) compared to the Vehicle group.

Figure B: Immobility time [s] for Vehicle and Oleamide treatments. The data is presented for two different groups: n=9 and n=10. Significant differences are indicated by symbols (###, **, *) compared to the Vehicle group.
Table 1. Effect of a combined intraperitoneal administration of (A) oleamide or (B) AM251 and antidepressant drugs on the spontaneous locomotor activity of mice

<table>
<thead>
<tr>
<th>Treatment (n=number of mice per group)</th>
<th>Travelled distance (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) vehicle + vehicle (n=8)</td>
<td>532.3 ± 46.99</td>
</tr>
<tr>
<td>oleamide + vehicle (n=8)</td>
<td>470.1 ± 64.35</td>
</tr>
<tr>
<td>imipramine + vehicle (n=8)</td>
<td>471.0 ± 51.25</td>
</tr>
<tr>
<td>imipramine + oleamide (n=8)</td>
<td>314.7 ± 62.99</td>
</tr>
<tr>
<td>escitalopram + vehicle (n=7)</td>
<td>699.7 ± 43.77</td>
</tr>
<tr>
<td>escitalopram + oleamide (n=8)</td>
<td>602.5 ± 98.12</td>
</tr>
<tr>
<td>reboxetine + vehicle (n=8)</td>
<td>460.1 ± 59.50</td>
</tr>
<tr>
<td>reboxetine + oleamide (n=8)</td>
<td>291.5 ± 68.27</td>
</tr>
<tr>
<td>(B) vehicle + vehicle (n=8)</td>
<td>458.9 ± 74.67</td>
</tr>
<tr>
<td>AM251 + vehicle (n=8)</td>
<td>477.2 ± 44.43</td>
</tr>
<tr>
<td>imipramine + vehicle (n=8)</td>
<td>449.1 ± 53.33</td>
</tr>
<tr>
<td>imipramine + AM251 (n=8)</td>
<td>472.1 ± 53.82</td>
</tr>
<tr>
<td>escitalopram + vehicle (n=8)</td>
<td>711.9 ± 70.70</td>
</tr>
<tr>
<td>escitalopram + AM251 (n=8)</td>
<td>724.2 ± 75.04</td>
</tr>
<tr>
<td>reboxetine + vehicle (n=8)</td>
<td>434.6 ± 56.76</td>
</tr>
<tr>
<td>reboxetine + AM251 (n=8)</td>
<td>630.0 ± 101.7</td>
</tr>
</tbody>
</table>

Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas oleamide (5 mg/kg) and AM251 (0.25 mg/kg) were given 30 min before the experiment. The values represent mean ± SEM.
**Table 2.** Effect of oleamide and AM251 on the brain levels of antidepressants in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug level in the brain (ng/g)</th>
<th>Treatment</th>
<th>Drug level in the brain (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>imipramine + vehicle (n=10)</td>
<td>2342 ± 169.0</td>
<td>imipramine + vehicle (n=10)</td>
<td>4020 ± 327.8</td>
</tr>
<tr>
<td>imipramine + oleamide (n=10)</td>
<td>3227 ± 380.5^</td>
<td>imipramine + AM251 (n=10)</td>
<td>3716 ± 378.4</td>
</tr>
<tr>
<td>desipramine + vehicle (n=10)</td>
<td>402.7 ± 26.27</td>
<td>desipramine + vehicle (n=10)</td>
<td>393.0 ± 34.74</td>
</tr>
<tr>
<td>desipramine + oleamide (n=10)</td>
<td>398.8 ± 11.38</td>
<td>desipramine + AM251 (n=10)</td>
<td>416.1 ± 74.22</td>
</tr>
<tr>
<td>escitalopram + vehicle (n=10)</td>
<td>412.1 ± 38.97</td>
<td>escitalopram + vehicle (n=6)</td>
<td>55.64 ± 7.746</td>
</tr>
<tr>
<td>escitalopram + oleamide (n=10)</td>
<td>388.0 ± 46.22</td>
<td>escitalopram + AM251 (n=8)</td>
<td>40.98 ± 9.970</td>
</tr>
<tr>
<td>reboxetine + vehicle (n=10)</td>
<td>160.5 ± 9.521</td>
<td>reboxetine + vehicle (n=10)</td>
<td>78.92 ± 5.651</td>
</tr>
<tr>
<td>reboxetine + oleamide (n=10)</td>
<td>155.6 ± 14.45</td>
<td>reboxetine + AM251 (n=10)</td>
<td>70.16 ± 9.093</td>
</tr>
</tbody>
</table>

Imipramine (15 mg/kg), escitalopram (2 mg/kg), and reboxetine (2.5 mg/kg) were injected 60 min before testing, whereas oleamide (5 mg/kg) AM251 (0.25 mg/kg) was given 30 min before decapitation. The values represent mean ± SEM (n=number of mice per group). ^p<0.05 versus imipramine (t-test)