Influence of the CB₁ and CB₂ cannabinoid receptor ligands on the activity of atypical antidepressant drugs in the behavioural tests in mice

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ABSTRACT

Available data support the notion that cannabinoids, whose therapeutic value is limited due to severe adverse reactions, could be beneficial as adjunctive agents in the management of mood disorders. Polytherapy, which is superior to monotherapy in the terms of effectiveness, usually requires lower doses of the individual components. Therefore, the main objective of our study was to determine whether administration of cannabinoid (CB) receptor ligands would enhance the antidepressant activity of atypical antidepressant drugs, i.e. agomelatine and tianeptine. To evaluate the antidepressant-like potential of the tested combinations, the mouse forced swim test (FST) and the tail suspension test (TST) were used. The HPLC method was applied to assess the brain levels of agomelatine and tianeptine. Both behavioural tests demonstrated that per se an ineffective intraperitoneal dose of oleamide (CB₁ receptor agonist, 5 mg/kg) potentiated the anti-immobility activity of tianeptine (15 mg/kg), whereas AM251 (CB₁ receptor inverse agonist/antagonist, 0.25 mg/kg) enhanced the antidepressant effects of tianeptine and agomelatine (20 mg/kg). Intraperitoneal co-administration of per se inactive doses of AM630 (CB₂ receptor inverse agonist/antagonist) and agomelatine or tianeptine significantly reduced the immobility time of animals only in the FST. CB receptor ligands did not affect the brain levels of the tested atypical antidepressants. In summary, the outcomes of the present study showed that activation and inhibition of CB₁ receptors as well as inhibition of CB₂ receptors may increase the antidepressant activity of tianeptine, whereas only inhibition of CB₁ and CB₂ receptors has a potential to augment the antidepressant activity of agomelatine.

1. Introduction

Since augmentation and combining therapies have become a common practice in management of the difficult-to-treat depression, new treatment strategies are being searched for. Available data support the notion that adjunctive use of agents modulating different neurochemical pathways involved in depression with antidepressant drugs may alleviate disease symptoms more profoundly than typical antidepressant monotherapy (Ceskova, 2016). Compounds with novel mechanisms of action are particularly under active investigation. Cannabinoids, i.e. ligands of cannabinoid (CB) receptors, belong to these substances. It was confirmed a long time ago that CB receptors play an important role in regulation of the excitatory (glutamate-related) and inhibitory (GABA-related) neurotransmissions in the brain as well as they modulate synthesis and release of monoamines (i.e., dopamine, norepinephrine, serotonin) (Moreira et al., 2009). Literature data also provide reliable evidence that the endocannabinoid system interplays with the hypothalamic–pituitary–adrenal axis, neuroplasticity markers (i.e., the brain-derived neurotrophic factor, BDNF), immune system, or acetylcholine pathways (McLaughlin et al., 2009; Moreira et al., 2009; Zoppi et al., 2014; Kruk-Słomka et al., 2015). All of the above-mentioned signalings are implicated in the pathomechanism of depression. Moreover, under certain conditions, CB receptor ligands display the anxiolytic-like activity (Moreira et al., 2009) and they produce rapid

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2. Materials and methods

The experiments were carried out in accordance with binding law related to studies on animal models as well as in compliance with the protocol approved by the Local Ethics Committee.

2.1. Animals

Drug and test naïve adult male Albino Swiss mice (8–10 weeks old, 25–30 g) provided by the Centre for Experimental Medicine (OMD) at the Medical University of Lublin were used in the study. Animals were kept in standard cages (8 individuals/cage) in the environmentally controlled facility, i.e. 12 h day/night cycle, temperature of 22–23 °C, humidity of 45–55%, with free access to both water and food. The bedding was corn cob granules and it was changed once a week. The refinement principle minimizing potential distress and enhancing animal welfare was applied. Overall 356 mice were randomly assigned to experimental groups. Each testing group was represented by 7–10 animals, depending on the research schedule. All behavioural experiments were performed between 8:00 and 15:00.

2.2. Drugs

The following agents were tested in the study: (i) CB1 receptor ligands – oleamide (cis-9,10-octadecenoamide, Tocris, USA) and AM251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, Tocris, USA), (ii) CB2 receptor ligands – JWH133 (6αR,10αR)-3-(1,1-Dimethylbutyl)-6a,7,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran, Tocris, USA) and AM630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl]4-methoxy-phenyl)methanone, Tocris, USA), and (iii) atypical antidepressants – agomelatine (Sigma-Aldrich, USA) and tianeptine (Sigma-Aldrich, USA). The CB receptor ligands and agomelatine were suspended in an aqueous solution of Tween 80 (1%), whereas tianeptine was dissolved in saline. Prepared suspensions and the solution were given as intraperitoneal (ip) injections: agomelatine and tianeptine – 60 min before testing, and oleamide, AM251, JWH133, and AM630 – 30 min before testing. The control animals received ip injections of vehicles, i.e. saline and the aqueous solution of Tween 80 (1%). The tested doses as well as the pretreatment schedules were chosen on the basis of the results of our previous projects (e.g., Szopa et al., 2019) and the literature data (Kruk-Słonka et al., 2015).

The following experimental groups were tested:

(i) the control group that received vehicles, i.e. saline + aqueous solution of Tween 80 (1%)
(ii) animals that received oleamide (5 mg/kg) + saline
(iii) animals that received AM251 (0.25 mg/kg) + saline
(iv) animals that received JWH133 (0.25 mg/kg) + saline
(v) animals that received AM630 (0.25 mg/kg) + saline
(vi) animals that received agomelatine (20 mg/kg) + saline
(vii) animals that received tianeptine (15 mg/kg) + aqueous solution of Tween 80 (1%)
(viii) animals that received oleamide (5 mg/kg) + agomelatine (20 mg/kg)
(ix) animals that received oleamide (5 mg/kg) + tianeptine (15 mg/kg)
(x) animals that received AM251 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xi) animals that received AM251 (0.25 mg/kg) + tianeptine (15 mg/kg)
(xii) animals that received JWH133 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xiii) animals that received JWH133 (0.25 mg/kg) + tianeptine (15 mg/kg)
(xiv) animals that received AM630 (0.25 mg/kg) + agomelatine (20 mg/kg)
(xv) animals that received AM630 (0.25 mg/kg) + tianeptine (15 mg/kg)

Scientific justification for carrying out the presented research was based on our previous reports (Poleszak et al., 2019) as well as on the findings of Takahashi et al. (2008) which demonstrated that CB receptor ligands are able to potentiate the activity of common antidepressant drugs that influence the monoaminergic neurotransmission. The following experimental groups were tested:

(i) the control group that received vehicles, i.e. saline + aqueous solution of Tween 80 (1%)
(ii) animals that received oleamide (5 mg/kg) + saline
(iii) animals that received AM251 (0.25 mg/kg) + saline
(iv) animals that received JWH133 (0.25 mg/kg) + saline
(v) animals that received AM630 (0.25 mg/kg) + saline
(vi) animals that received agomelatine (20 mg/kg) + saline
(vii) animals that received tianeptine (15 mg/kg) + aqueous solution of Tween 80 (1%)

The experiments were carried out in accordance with binding law related to studies on animal models as well as in compliance with the protocol approved by the Local Ethics Committee.

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behavioural responses (Linge et al., 2016). Unfortunately, both stimulation and inhibition of the endocannabinoid system (particularly via CB1 receptors) entail the development of severe adverse reactions (like agitation, aggression, eating disorders, seizures, hypertension, emesis, hypokalaemia) that limit the therapeutic value of CB receptor ligands in monotherapy (Moreira and Crippa, 2009; Hermanns-Clausen et al., 2013). However, CB receptor ligands are still regarded as compounds with a huge therapeutic potential in polytherapy. Polytherapy, which usually requires lower doses of the individual components, could be at least equal to monotherapy in the terms of effectiveness.

Therefore, in the present study we decided to investigate whether CB receptor ligands would enhance the antidepressant activity of atypical antidepressant drugs, i.e. agomelatine and tianeptine are clinically used as a part of combined therapy with standard antidepressant drugs, and such a polytherapy seems to be a more favourable strategy than monotherapy, particularly in the treatment-resistant cases (Tobe and Rybakowski, 2013; Fastie, 2019; Potměšil, 2019).
2.3. Forced swim test (FST)

The FST was carried out according to the procedure described by Porsolt et al. (1977). Briefly, each mouse was suspended by the tail (2 cm from the end of the tail) about 50 cm above the floor, using an adhesive tape. It was left there for 6 min. Immobility of animals, i.e. duration of time when a suspended mouse stopped struggling in the air and performed only movements necessary to breathe, was measured between the 2nd and 6th min of the test.

2.4. Tail suspension test (TST)

The TST was carried out according to the procedure described by Steru et al. (1985). Each mouse was suspended by the tail (2 cm from the end of the tail) about 50 cm above the floor, using an adhesive tape. It was left there for 6 min. Immobility of animals, i.e. duration of time when a suspended mouse stopped struggling in the air and performed only movements necessary to breathe, was measured between the 2nd and 6th min of the test.

2.5. Spontaneous locomotor activity

Measurements of spontaneous locomotor activity was carried out according to the procedure we had used before (Szopa et al., 2019). An activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, USA) was used. The device consists of 4 transparent cages with lids equipped with 4 infrared emitters with laser beams and 4 detectors monitoring animal movements. Each mouse was placed individually into the cage (43 cm × 43 cm × 32 cm) and left there for 6 min. A distance travelled by a given animal was recorded automatically during the time interval corresponding to the one analyzed in the FST and the TST, that is between the 2nd and the 6th min of the test.

2.6. Determination of the brain levels of agomelatine and tianeptine

60 min following injection of a given atypical antidepressant (with or without a CB receptor ligand), the tested animals were decapitated and their brains were collected and frozen. The brain levels of agomelatine and tianeptine were assessed by the high-performance liquid chromatography (HPLC) method, according to the procedure we had used before (Szopa et al., 2019). The brains were homogenized in distilled water (1:4, w/v) with a TH220 tissue homogenizer (Omni International, Inc., Warrenton, VA, USA). For agomelatine, 1 ml of brain homogenate was spiked with carbamazepine (100 ng/ml) as an internal standard (IS). Before the extraction, 1 ml of the concentrated NaCl solution (10 g/50 ml) was added to brain homogenate and the samples were vortexed for 15 s. The extraction of agomelatine from brain homogenate was performed using 5 ml of a mixture of dichloromethane/hexane/isooamyl alcohol (39.5:59.5:1 v/v/v). After centrifugation, the organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C under a gentle stream of nitrogen in a water bath. The residues were dissolved with 100 μl of methanol, and aliquots of 50 μl were injected into the HPLC system.

The HPLC system consisted of an isocratic pump (model L-7100) and an autosampler (model L-7200), both from Merck Hitachi (Darmstadt, Germany), and a UV variable-wavelength K-2600 detector (Knauer, Berlin, Germany). Data acquisition and processing were carried out using the D-7000 HSM software (Merck Hitachi). Analysis of agomelatine and tianeptine was performed on a 250 × 4 mm LiChrospher1100 RP-18 column with a particle size of 5 μm (Merck, Darmstadt, Germany) protected with a guard column (4 × 4 mm) with the same packing material. The mobile phase consisting of acetonitrile and 50 mM potassium dihydrogen phosphate was mixed at a ratio of 37:63 (v/v) for agomelatine and 31:69 (v/v) for tianeptine and run at 1 ml/min. Chromatographic analysis was carried out at 21 °C and an analytical wavelength of 230 nm for agomelatine and 214 nm for tianeptine.

The calibration curves constructed by plotting the ratio of the peak heights of the studied drug to IS versus the concentration of the drug were linear in the tested concentration ranges. No interfering peaks were observed in the chromatograms. The measurements were reproducible with low intra- and inter-day variation (coefficient of variation < 10%). The extraction efficiencies of the analyzed compounds and the internal standard ranged from 70% to 95%. Levels of the tested antidepressants were given in ng/g (for the wet brain tissue).

2.7. Statistical analysis

Statistical analysis was performed either by two-way analysis of variance (ANOVA) with Bonferroni’s post-hoc test or by t-test, depending on the study design. The results from the behavioural tests were calculated by two-way ANOVA, whereas the outcomes from the pharmacokinetic analyses were calculated by t-test. In two-way ANOVA, the following independent variables were taken into consideration: (i) treatment with an atypical antidepressant, and (ii) treatment with a CB receptor ligand. The results were presented as the means ± standard error of the mean (SEM). Between-group differences with p lower than 0.05 were treated as statistically significant (where: *p < 0.05, **p < 0.01, and ***p < 0.001).

3. Results

An acute ip injection of oleamide (5 mg/kg), AM251 (0.25 mg/kg), JWH133 (0.25 mg/kg), AM630 (0.25 mg/kg), agomelatine (20 mg/kg), or tianeptine (15 mg/kg) did not change the immobility time of the tested mice in the FST and in the TST, when compared to the vehicle-treated group.

3.1. Effects of a concomitant administration of oleamide and the atypical antidepressants in the FST and the TST

As presented in Fig. 1, an acute injection of oleamide (5 mg/kg) did not potentiate the activity of agomelatine given at a dose of 20 mg/kg either in the FST or in the TST. Though Bonferroni’s post-hoc test detected significant differences in the FST between the group that received oleamide + agomelatine and the group that received only agomelatine, two-way ANOVA demonstrated a non-significant oleamide-agomelatine interaction in the FST [F(1,35) = 1.07; p = 0.3074] and also a non-significant oleamide-agomelatine interaction in the TST [F(1,36) = 1.03; p = 0.3178]. On the other hand, oleamide managed to augment the anti-immobility effect of tianeptine (15 mg/kg) in both applied tests. Statistical analysis by two-way ANOVA confirmed a significant oleamide-tianeptine interaction in the FST [F(1,34) = 5.68; p = 0.0229] with a significant effect of oleamide [F(1,34) = 18.72; p = 0.0001] and a significant effect of tianeptine [F(1,34) = 6.01; p = 0.0195]. Similarly, two-way ANOVA demonstrated a significant oleamide-tianeptine interaction in the TST [F(1,36) = 17.43; p = 0.0002] with a significant effect of oleamide [F(1,36) = 10.71; p = 0.0024] and a significant effect of tianeptine [F(1,36) = 12.21;
After combined administration of AM251 (0.25 mg/kg) and agomelatine (20 mg/kg) or tianeptine (15 mg/kg), the tested mice struggled for a longer time when placed in the water or suspended by their tails in comparison to the animals that were given the respective monotherapy (Fig. 2). Two-way ANOVA revealed: (1) a significant AM251-agomelatine interaction \[F(1,28) = 5.61; p = 0.0250\] in the FST, with a significant effect of AM251 \[F(1,28) = 23.29; p < 0.0001\] and a significant effect of agomelatine \[F(1,28) = 8.61; p = 0.0066\], (2) a significant AM251-agomelatine interaction \[F(1,26) = 8.23; p = 0.0081\] in the TST, with a significant effect of agomelatine \[F(1,26) = 5.75; p = 0.0240\] but a not significant effect of AM251 \[F(1,26) = 0.91; p = 0.3499\], (3) a significant AM251-tianeptine interaction \[F(1,26) = 4.33; p = 0.0475\] in the FST, with a significant effect of AM251 \[F(1,26) = 9.06; p = 0.0057\] and a significant effect of tianeptine \[F(1,26) = 20.64; p = 0.0001\], (4) a significant AM251-tianeptine interaction \[F(1,28) = 11.40; p = 0.0022\] in the TST, with a significant effect of tianeptine \[F(1,28) = 26.95; p < 0.0001\] but a not significant effect of AM251 \[F(1,28) = 2.17; p = 0.1521\].

3.3. Effects of a concomitant administration of JWH133 and the atypical antidepressants in the FST and the TST

Addition of JWH133 (0.25 mg/kg) to the treatment with agomelatine (20 mg/kg) or tianeptine (15 mg/kg) did not potentiate the activity of the tested atypical antidepressants in either of the applied tests. The animals that received JWH133 + agomelatine or JWH133 + tianeptine behaved almost in the same manner in the FST and in the TST as the mice that received only JWH133 or the respective antidepressant drug. Though Bonferroni's post-hoc test detected significant differences in the FST between the group that received JWH133 + tianeptine and the groups that received only JWH133 or the vehicle, two-way ANOVA did not detect any significant drug-drug interaction for the JWH133-tianeptine treatment in the FST \(F(1,28) = 1.03; p = 0.3182\) or in the TST \(F(1,28) = 1.02; p = 0.3211\). Similarly, a non-significant interaction was obtained for the JWH133-agomelatine treatment in the FST \(F(1,28) = 2.73; p = 0.1094\) and in the TST \(F(1,28) = 0.08; p = 0.7846\). The results were illustrated in Fig. 3.
3.4. Effects of a concomitant administration of AM630 and the atypical antidepressants in the FST and the TST

Mice that received a combination of AM630 (0.25 mg/kg) and agomelatine (20 mg/kg) or tianeptine (15 mg/kg) were actively moving for a longer time in the FST in comparison to animals from the control groups. Such an effect was not observed in the TST – mice treated with a given concomitant therapy stayed immobile for a similar duration of time as the animals subjected to the respective monotherapy (Fig. 4). Calculations with two-way ANOVA showed a significant AM630-agomelatine interaction \( F(1,28) = 13.26; p = 0.0011 \) in the FST, with a significant effect of AM630 \( F(1,28) = 14.32; p = 0.0007 \) and a significant effect of agomelatine \( F(1,28) = 7.05; p = 0.0129 \), but a not significant AM630-agomelatine interaction \( F(1,28) = 3.45; p = 0.0736 \) in the TST. Correspondingly, according to the statistical outcomes, AM630-tianeptine interaction in the FST was significant \( F(1,27) = 4.98; p = 0.0341 \), with a significant effect of both AM630 \( F(1,27) = 5.75; p = 0.0237 \) and tianeptine \( F(1,27) = 7.19; p = 0.0124 \), but it was not significant in the TST \( F(1,28) = 1.67; p = 0.2071 \).

3.5. Effects of a concomitant administration of the CB receptor ligands and the atypical antidepressants on the spontaneous locomotor activity of mice

None of the tested agents (i.e., oleamide, AM251, JWH133, AM630, agomelatine, and tianeptine) or their respective combinations significantly increased the spontaneous locomotor activity of mice (Table 1).

3.6. Brain levels of agomelatine and tianeptine

In the statistical analysis of outcomes from the pharmacokinetic assay, we took into consideration only these combinations that had acted synergistically in the behavioural tests, i.e. AM251 (0.25 mg/kg) or AM630 (0.25 mg/kg) with agomelatine (20 mg/kg), and oleamide (5 mg/kg), AM251, or AM630 with tianeptine (15 mg/kg). As presented in Table 2, none of the tested CB receptor ligands increased or reduced the brain levels of the atypical antidepressants. Calculations with \( t \)-test gave the following results: (1) \( t(14) = 0.1431, p = 0.8882 \) for the AM251-agomelatine combination, (2) \( t(13) = 1.895, p = 0.0806 \) for the AM630-agomelatine combination, (3) \( t(18) = 0.4003, p = 0.6937 \) for the oleamide-tianeptine combination, (4) \( t(14) = 1.104, p = 0.2882 \) for the AM251-tianeptine combination, and (5) \( t(14) = 0.8493, p = 0.4100 \) for the AM630-tianeptine combination.
whereas it was not a

towards CB1 receptors, i.e. by administration of per se ine

inhibition of either CB1 or CB2 receptor functioning. Stimulation of

0.25 mg/kg). As for the activity of agomelatine, it was enhanced only

verse agonist/antagonist of CB 2 receptors (AM630, 0.25 mg/kg),

We demonstrated that the antidepressant e

interaction between CB1 and CB2 receptor ligands and atypical anti-

4. Discussion

administered intraperitoneally 30 min before decapitation, whereas tianeptine

(15 mg/kg) and agomelatine (20 mg/kg) were injected intraperitoneally 60 min

Table 1

A

B

C

D

Vehicle + vehicle (n = 8)

Vehicle + vehicle (n = 8)

Vehicle + vehicle (n = 8)

Vehicle + vehicle (n = 8)

Oleamide + vehicle (n = 8)

AM251 + vehicle (n = 8)

Agomelatine + vehicle (n = 8)

Tianeptine + vehicle (n = 8)

Agomelatine + oleamide (n = 7)

Tianeptine + oleamide (n = 7)

Agomelatine + oleamide (n = 7)

Tianeptine + vehicle (n = 8)

Agomelatine + AM251 (n = 8)

Tianeptine + vehicle (n = 8)

Tianeptine + AM251 (n = 8)

Agomelatine + vehicle (n = 8)

JWH133 + vehicle (n = 8)

JWH133 + AG251 (n = 8)

Ve...
receptor ligands, modulation of the serotonin-, glutamate-, immune-, and/or neurotrophin-related pathways may be involved in their antidepressant-like activity (Benito et al., 2008; García-Gutiérrez et al., 2010; Zoppi et al., 2014; Işığuro et al., 2018). Therefore, we assume that the enhancement of the serotonergic neurotransmission may be particularly responsible for the observed interactions between CB receptor ligands and agomelatine, whereas potentiation of the dopaminergic signaling may be mainly responsible for the positive interplay between CB receptor ligands and tianeptine. Keeping in mind that AM630 is not only an inverse agonist/antagonist of CB receptors but it also has an affinity towards CB receptors and it acts as an inverse agonist (Landsman et al., 1998), one can suspect that an interplay between AM630 and CB receptors can contribute to the final effects of AM630 treatment in our studies. We guess that co-administration of oleamide and agomelatine as well as concurrent use of JWH133 and agomelatine or tianeptine were unable to sufficiently increase the levels of monoamines, and this is the reason why these combinations did not induce shortening of the immobility time of animals in the applied behavioural tests. Of note is the fact that the antidepressant activity of agomelatine is partially due to the inhibition of serotonergic 5-HT2A receptors, whereas in studies by Franklin et al. (2013) JWH133 has emerged as an agent that upregulates these receptors. So, the opposite activity towards serotonergic 5-HT2A receptors may be responsible for the lack of the antidepressant potential of the JWH133-agomelatine combination.

Drug-drug interactions detected in our study are most probably due to mechanisms that take place at the cellular level, since our pharmako-cinetic studies did not reveal significant alterations in the brain levels of the tested atypical antidepressants after concomitant administration with CB receptor ligands. Based on the outcomes of Smaga et al. (2017), an acute administration of tianeptine could have increased CB1 density in different parts of the brain, including the motor cortex, frontal cortex, and hippocampus. However, we should not expect an intensification of the interactions have rather the pharmacodynamic background instead of the pharmacokinetic one. These preliminary findings need to be confirmed in further experiments, but it seems that the adjuvant therapy to atypical antidepressants based on modulation of the endocannabinoid system (particularly via CB2 receptors) could be a promising treatment option. Such a strategy may be beneficial in patients suffering from depression that co-exists with anxiety, since CB receptor ligands, agomelatine, and tianeptine have a certain anxiolytic potential (Pertwee, 2015; Brink et al., 2006; McEwen et al., 2010; Fasipe, 2019).

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**References**

Adamczyk, P., Golda, A., McCreary, A.C., Filip, M., Przegalinski, E., 2008. Activation of endocannabinoid transmission induces antidepressant-like effects in rats. J. Phyisol. Pharmacol. 59, 217–228.

Bambico, R., Cassano, T., Dominguez-Lopez, S., Katz, N., Walker, D.C., Piomelli, D., Gobbi, G., 2010. Genetic deletion of fatty acid amide hydrolase alters emotional behavior and serotonergic transmission in the dorsal raphe, prefrontal cortex, and hippocampus. Neuropsychopharmacology 35, 2085–2100.

Benito, G., Tolón, R.M., Paseo, M.R., Nunez, E., Castillo, A.I., Romero, J., 2008. Cannabinoid CB2 receptors in human brain inflammation. Br. J. Pharmacol. 153, 277–285.

Beyer, C.E., Dwyer, J.M., Piela, M.J., Platt, B.J., Shen, R., Rahman, Z., Chan, K., Manners, M.T., Samad, T.A., Kennedy, J.D., Bingham, B., Whiteside, G.T., 2010. Depression-like phenotype following chronic CBl receptor antagonism. Neurobiol. Dis. 39, 148–155.

Brint, C.B., Harvey, R.H., Brind, L., 2006. Tianeptine: a novel atypical antidepressant that may provide new insights into the biomolecular basis of depression. Recent CNS Drug Discov 1, 29–41.

Ceskova, E., 2016. Current pharmacotherapy of depression - focused on multimodal/multifunctional antidepressants. Expert. Opin. Pharmacother. 17, 1835–1837.

Cryan, J.F., Mombereau, C., Vassou, A., 2005. The tail suspension test as a model for assessing antidepressant activity; review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev. 29, 571–625.

Esethan, S., García-Sevilla, J.A., 2012. Effects induced by cannabinoids in monoaminergic systems in the brain and their implications for psychiatric disorders. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 38, 78–87.

Fasipe, O.J., 2019. The emergence of new antidepressants for clinical use: agomelatine paradox versus other novel agents. BBRO Rep 6, 95–110.

Franklin, J.M., Vasiljevik, T., Prisinzano, T.E., Carrasco, G.A., 2013. Depression-like syndrome in mice. Neurosci. Biobehav. Rev. 37, 760–767.

García-Gutiérrez, M.S., Perez-Ortiz, J.M., Gutierrez-Adan, A., Manzanares, J., 2010. Depression-resistant endophenotype in mice overexpressing cannabinoid CB2 (receptor) br. Br. J. Pharmacol. 160, 1773–1784.

Griebel, G., Stemmlein, J., Scatton, B., 2005. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol. Psychiatry 57, 261–267.

Guardiola-Lemaître, B., De, B.C., Delargrance, P., Millan, M.J., Munoz, C., Mocaer, E., 2014. Agomelatine: mechanism of action and pharmacological profile in relation to antidepressant properties. Br. J. Pharmacol. 171, 3604–3619.

Häring, M., Grieb, M., Monory, K., Lutz, B., Moreira, F.A., 2013. Cannabinoid CB(1) receptor in the modulation of stress coping behavior in mice: the role of serotonin and different forebrain neuronal subpopulations. Neuropharmacology 65, 83–89.

Hermens, G., Coen, M., Knoefel, C., Awartani, V., 2013. Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. Addiction 108, 534–544.

Işığuro, H., Horiiuchi, Y., Tabata, K., Liu, Q.R., Artimani, T., Onaivi, E.S., 2018. Cannabinoid CB2 receptor gene and environmental interaction in the development of psychiatric disorders. Molecules 23 (molecules23081836).

Khakpour, F., Ebrabhi-Mehr, I., Alijanpour, S., Zarrindast, M.R., 2019. Ketamine-induced antidepressant-like effects in mice: a possible involvement of cannabinoid system. Biomed. Pharmacother. 112, 106717.

Krul-Slomka, M., Michalak, A., Biela, G., 2015. Antidepressant-like effects of the cannabinoid receptor ligands in the forced swimming test in mice: mechanism of action and possible interactions with cholinergic system. Behav. Brain Res. 284, 24–36.

Landsman, R.S., Makriyannis, A., Deng, H., Conrow, P., Roese, W.R., Yamamura, H.I., 1998. AM630 is an inverse agonist at the human cannabinoid CB1 receptor. Life Sci. 62, L109-L113.

Linga, J., Jimenez-Sanchez, L., Campa, L., Pilar-Cuellar, F., Vital, R., Paseos, A., Adell, A., Diaz, A., 2016. Cannabidiol induces rapid-acting antidepressant-like effects and enhances corticosterone 5-HT/glutamate neurotransmission: role of 5-HT1A receptors. Neuropsychopharmacology 103, 16–26.

McEwen, B.S., Chattarji, S., Diamond, D.M., Jay, T.M., Reagan, L.P., Svenningsen, P., Fuchs, E., 2010. The neurobiological properties of tianeptine (Stablon): from monoamine hypothesis to glutamatergic modulation. Mol. Psychiatry 15, 237–249.

McLaughlin, R.J., Hill, M.N., Gorralka, B.B., 2009. Monosynaptic neurotransmission contributes to cannabinoid-induced activation of the hypothalamic-pituitary-adrenal axis. Eur. J. Pharmacol. 624, 71–76.

Moreira, F.A., Crippa, J.A., 2009. The psychiatric side-effects of rimonabant. Braz J Psychiatry 31, 145–153.

Moreira, F.A., Grieb, M., Lutz, B., 2009. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 23, 133–144.

Onaivi, E.S., Işığuro, H., Gong, J.P., Patel, S., Mozaffi, P., Myers, L., Perchak, A., Mora, Z., Tagliaferro, P.A., Gardner, E., Brusco, A., Akinsbola, B.E., Hope, B., Lujilde, J., 2018.
Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., Uhl, G.R., 2008. Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. PLoS One 20 (3), e1640.

Ostadhadi, S., Haj-Mirzaian, A., Nikoui, V., Kordjazy, N., Dehpour, A.R., 2016. Involvement of opioid system in antidepressant-like effect of the cannabinoid CB1 receptor inverse agonist AM-251 after physical stress in mice. Clin. Exp. Pharmacol. Physiol. 43, 203–212.

Patel, S., Hillard, C.J., 2009. Role of endocannabinoid signaling in anxiety and depression. Curr. Top. Behav. Neurosci. 1, 347–371.

Endocannabinoids. In: Pertwee (Ed.), Handbook of Experimental Pharmacology. Springer International Publishing, Switzerland.

Poleszak, E., Wosko, S., Sławińska, K., Wyska, E., Szopa, A., Doboszewska, U., Wlaz, P., Wlaz, A., Dudka, J., Szporaz, J., Serefko, A., 2019. Influence of the CB1 cannabinoid receptors on the activity of the monoaminergic system in the behavioural tests in mice. Brain Res. Bull. 150, 179–185.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Potměšil, P., 2019. What combinations of agomelatine with other antidepressants could be successful during the treatment of major depressive disorder or anxiety disorders in clinical practice? Ther Adv Psychopharmacol 9, 2045125319855206.

Shearman, L.P., Rosko, K.M., Fleischer, R., Wang, J., Xu, S., Tong, X.S., Rocha, B.A., 2003. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. Behav. Pharmacol. 14, 573–582.

Smaga, I., Zanierska, M., Gwulinski, D., Faron-Gorecka, A., Szafarzuk, P., Ceglą, M., Filip, M., 2017. Changes in the cannabinoids receptors in rats following treatment with antidepressants. Neurotoxicology 63, 13–20.

Steru, L., Chemat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Szopa, A., Bogatko, K., Sereńko, A., Wyska, E., Wosko, S., Szwiderska, K., Doboszewska, U., Wlaz, A., Wrobel, A., Wlaz, P., Dudka, J., Poleszak, E., 2019. Agomelatine and tianeptine antidepressant activity in mice behavioral despair tests is enhanced by DMPX, a selective adenosine A2A receptor antagonist, but not DPCPX, a selective adenosine A1 receptor antagonist. Pharmacol. Rep. 71, 676–681.

Takahashi, E., Katayama, M., Niimi, K., Itakura, C., 2008. Additive subthreshold dose effects of cannabinoid CB(1) receptor antagonist and selective serotonin reuptake inhibitor in antidepressant behavioral tests. Eur. J. Pharmacol. 589, 149–156.

Tobe, E.H., Rybakowski, J.K., 2013. Possible usefulness of tianeptine in treatment-resistant depression. Int. J. Psychiatry Clin. Pract. 17, 313–316.

Zoppì, S., Madrigal, J.L., Caso, J.R., Garcia-Gutierrez, M.S., Manzanares, J., Leza, J.C., Garcia-Bueno, R., 2014. Regulatory role of the cannabinoid CB2 receptor in stress-induced neuroinflammation in mice. Br. J. Pharmacol. 171, 2814–2826.