Toxicology and Applied Pharmacology 337 (2017) 95–103

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Chronic treatment with caffeine and its withdrawal modify the antidepressant-like activity of selective serotonin reuptake inhibitors in the forced swim and tail suspension tests in mice. Effects on *Comt*, *Slc6a15* and *Adora1* gene expression

Aleksandra Szopa[a,*], Urszula Doboszewska[b], Mariola Herbet[c], Sylwia Wośko[a], Elżbieta Wyska[d], Katarzyna Świąder[a], Anna Serefko[a], Agnieszka Korga[c], Aleksandra Wlaź[e], Andrzej Wróbel[f], Marta Ostrowska[c], Joanna Terlecka[a], Adam Kanadys[c], Ewa Poleszak[a], Jarosław Dudka[c], Piotr Wlaź[b]

[a] Chair and Department of Applied Pharmacy, Medical University of Lublin, Lublin, Poland
[b] Department of Animal Physiology, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Lublin, Poland
[c] Chair and Department of Toxicology, Medical University of Lublin, Lublin, Poland
[d] Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, Kraków, Poland
[e] Department of Pathophysiology, Medical University of Lublin, Lublin, Poland
[f] Second Department of Gynecology, Medical University of Lublin, Lublin, Poland

ARTICLE INFO

Keywords:
Depression
Caffeine
SSRI
Slc6a15
Adora1
Mice

ABSTRACT

Recent preclinical and clinical data suggest that low dose of caffeine enhances the effects of common antidepressants. Here we investigated the effects of chronic administration of caffeine (5 mg/kg, twice daily for 14 days) and its withdrawal on day 15th on the activity of per se ineffective doses of fluoxetine (5 mg/kg) and escitalopram (2 mg/kg) given on day 15th. We found decreased immobility time in the forced swim and tail suspension tests in mice in which caffeine was administered simultaneously with antidepressants on day 15th following a 14-day caffeine treatment and no alterations in the spontaneous locomotor activity. A decrease in the level of escitalopram and an increase in the level of caffeine in serum were observed after concomitant administration of these compounds, while the joint administration of caffeine and fluoxetine was not associated with changes in their levels in serum or brain. Caffeine withdrawal caused a decrease in *Adora1* mRNA level in the cerebral cortex (Cx). Administration of escitalopram or fluoxetine followed by caffeine withdrawal caused an increase in this gene expression, whereas administration of escitalopram, but not fluoxetine, on day 15th together with caffeine caused a decrease in *Adora1* mRNA level in the Cx. Furthermore, antidepressant-like activity observed after joint administration of the tested drugs with caffeine was associated with decreased *Slc6a15* mRNA level in the Cx. The results show that withdrawal of caffeine after its chronic intake may change activity of antidepressants with concomitant alterations within monoamine, adenosine and glutamate systems.

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is the most frequently consumed psychoactive stimulant substance in the world (Heckman et al., 2010; Nehlig, 1999; El Yacoubi et al., 2003). Nearly all amount of the ingested caffeine derives from the dietary sources, particularly from coffee, tea, cola, and energy drinks (Fredholm et al., 1999). The most significant behavioral effects occur after low to moderate doses (50–300 mg) of caffeine (Benowitz, 1990). At the doses usually consumed by people (i.e., one/two cups of coffee per day) caffeine acts as a non-selective antagonist of the adenosine receptors designated as A_1 and A_{2A} subtypes (Fredholm, 1995; Fredholm et al., 2001; Fredholm et al., 2011; Fredholm et al., 2016), whereas the larger quantities of caffeine lead to the release of intracellular calcium ions and inhibition of cyclic

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); *Adora1*, adenosine A_1 receptor gene; *Comt*, catechol-*O*-methyltransferase gene; Cx, cerebral cortex; DA, dopamine; FST, forced swim test; GABA, γ-aminobutyric acid; HPLC, high-performance liquid chromatography; i.p., intraperitoneally; IS, internal standard; NA, noradrenaline; S.E.M., standard error of the mean; *Slc6a15*, solute carrier family 6 member 15 gene; SSRI, selective serotonin reuptake inhibitor; TST, tail suspension test

* Corresponding author at: Chair and Department of Applied Pharmacy, Medical University of Lublin, Chodźki 1, PL 20–093 Lublin, Poland.
E-mail address: aleksandra.szopa@umlub.pl (A. Szopa).

http://dx.doi.org/10.1016/j.taap.2017.10.020
Received 5 July 2017; Received in revised form 20 October 2017; Accepted 24 October 2017
Available online 09 November 2017
0041-008X/

nucleotide phosphodiesterases (Nehlig et al., 1992; Nehlig and Debry, 1994). Because of its inhibitory action on the adenosine receptors, caffeine is known to increase the levels of neurotransmitters in the central nervous system (CNS), such as noradrenaline (NA) and dopamine (DA) (Daly and Fredholm, 1998; Kale et al., 2010). Chronic administration of common antidepressant drugs likewise enhances the concentration of neurotransmitters (including serotonin (5-HT), NA, and DA) (Nutt, 2006), thus improving the mood (Kale et al., 2010). Because caffeine is a well-known CNS stimulant it could influence affective disorders, such as depression (Garcia-Blanco et al., 2016).

In patients with depression, symptoms such as fatigue, tiredness or diminished activity are common (Vaccarino et al., 2016). Therefore, an increased intake of caffeine in these individuals in comparison to healthy controls has been observed (Leibenluft et al., 1993; Rihs et al., 1996; Whalen et al., 2008). In several studies an inverse correlation between high caffeine consumption and a risk of depression and suicide has been shown (Lucas et al., 2011; Ruusunen et al., 2010; Lucas et al., 2014). Recent study demonstrated that chronic supplementation with low dose of caffeine produced rapid (at 2 weeks of treatment) antidepressant action in patients treated with the selective serotonin reuptake inhibitor (SSRI) escitalopram (Liu et al., 2017). The available preclinical data have shown an interaction between caffeine and commonly used antidepressants after administration of single doses of each (Kale and Addepalli, 2014; Robles-Molina et al., 2012; Szopa et al., 2016; Poleszak et al., 2016; Poleszak et al., 2015; Serefko et al., 2016). Since caffeine is usually consumed regularly (i.e., 2–3 times per day) and based on clinical report showing positive results of chronic caffeine administration in depressed patients treated with an SSRI, here we tested the effects of chronic administration of caffeine and its withdrawal on the activity of generally prescribed antidepressants belonging to the SSRIs, namely, fluoxetine and escitalopram, in the forced swim test (FST) and the tail suspension test (TST) in mice. To evaluate whether the observed effects on animal behavior were due to a pharmacokinetic or pharmacodynamic interaction, the levels of the studied antidepressants and caffeine in mice serum and brain tissue were measured using the high-performance liquid chromatography (HPLC) methods. Moreover, the relative expression of the selected genes, for which the role in the pathophysiology and treatment of depression and/or the mechanism of action of caffeine has been suggested: adenosine A_1 receptor (*Adora1*), encoding A_1 receptor; solute carrier family 6 member 15 (*Slc6a15*), encoding a transporter belonging to neutral amino acid transporters subfamily of the *Slc6* family (Pramod et al., 2013), which is characterized by the Na^+-dependent translocation of amino acids such as proline, which can be further used in the biosynthesis of the neurotransmitter glutamate (Santarelli et al., 2015) and catechol-*O*-methyltransferase (*Comt*), encoding enzyme responsible for degradation of catecholamine neurotransmitters was assessed using a quantitative real-time PCR (qRT-PCR) assay.

2. Materials and methods

2.1. Animals

The experiment was carried out on naïve adult male Albino Swiss mice (25–30 g) purchased from the licensed breeder (Kołacz, Warsaw, Poland). The animals were housed in the environmentally controlled rooms with a 12 h light/dark cycle, in groups of 10 in standard cages under strictly controlled laboratory conditions – temperature maintained at 22–23 °C with relative humidity in a range of 45–55%. Throughout the study, the animals were given ad libitum access to water and food. The experiment began after at least a 1-week acclimation period to the laboratory conditions and was conducted between 8 a.m. and 3 p.m. to minimize circadian influences. Each experimental group consisted of 8–10 animals. Procedures involving mice and their care in all the experiments of the present study were approved by the Local Ethics Committee at the Medical University of Lublin (license no 29/2015) and were performed in accordance with binding European standards related to the experimental studies on animal models. Each mouse was used only once.

2.2. Drug administration

Caffeine (5 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), fluoxetine hydrochloride (5 mg/kg, Sigma-Aldrich), and escitalopram oxalate (2 mg/kg, Sigma-Aldrich), were dissolved in saline (0.9% NaCl solution) immediately prior to the intraperitoneal (i.p.) administration. The doses and treatment schedules were selected on the basis of the literature data (Gasior et al., 1996) and confirmed in our previous experiments (Szopa et al., 2016). The volume of saline or caffeine/antidepressant solutions for i.p. administration was 10 ml/kg.

2.3. Treatment protocol

The solution of caffeine was injected i.p. twice a day (at 08.00 a.m. and 08.00 p.m.) for 14 days, and on the 15th day it was administered 40 min before behavioral testing, whereas the solutions of antidepressants were given i.p. once – on the 15th day, 60 min before the experiment, according to the following scheme: (I) caffeine (10 mg/kg/day) for 14 days + saline on the 15th day; (II) caffeine (10 mg/kg/day) for 14 days + caffeine (5 mg/kg) on the 15th day; (III) caffeine (10 mg/kg/day) for 14 days + fluoxetine (5 mg/kg) on the 15th day; (IV) caffeine (10 mg/kg/day) for 14 days + caffeine (5 mg/kg) and fluoxetine (5 mg/kg) on the 15th day; (V) caffeine (10 mg/kg/day) for 14 days + escitalopram (2 mg/kg) on the 15th day; (VI) caffeine (10 mg/kg/day) for 14 days + caffeine (5 mg/kg) and escitalopram (2 mg/kg) on the 15th day.

2.4. Forced swim test (FST)

The procedure was carried out according to the method of Porsolt et al. (1977). Each mouse was placed individually into the 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 total duration of immobility was recorded during the last 4 min of the 6-min long testing period. The mouse was judged immobile when it ceased struggling and remained floating motionless in the water, making only the movements necessary to keep its head above the water level. The immobility time was scored in real time by two blind observers.

The results obtained in the FST are shown as the arithmetic mean of immobility time of animals given in seconds ± standard error of the mean (S.E.M.) for each experimental group.

2.5. Tail suspension test (TST)

The procedure was carried out according to the method of Steru et al. (1985). Each mouse was individually suspended by its tail to a vertical bar in wooden box (30 × 30 cm). The animals were fastened by means of an adhesive tape fixed 2 cm from the end of the tails for 6 min. The total duration of immobility was recorded during the last 4 min of the 6-min long testing period. The mouse was judged immobile when it ceased moving its limbs and body, making only the movements necessary to breathe. The immobility time was scored in real time by two blind observers.

The results obtained in the TST are shown as the arithmetic mean of immobility time of animals given in seconds ± S.E.M. for each experimental group.

2.6. Spontaneous locomotor activity

The spontaneous locomotor activity was measured in an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, Columbus, OH, USA). This device consists of four transparent cages

with a lid (43 × 43 × 32 cm), a set of four infrared emitters (each emitter has 16 laser beams), and four detectors monitoring animal movements. Mice were placed individually into the cages for 10 min. Spontaneous locomotor activity was evaluated between the 2nd and the 6th minute, which corresponds to the time interval analyzed in the FST and TST.

The results obtained in this test are presented as the arithmetic average distance traveled by a mouse in centimeters ± S.E.M. for each experimental group.

2.7. Determination of tested substances in serum and brain homogenates

At the appropriate time points after chronic administration of caffeine and a single dose of the antidepressant drugs given alone or simultaneously with caffeine, mice were decapitated to collect biological material for pharmacokinetic studies. The blood was collected into Eppendorf tubes and allowed to clot at room temperature. Subsequently, the blood was centrifuged at 10,000 rpm for 10 min and serum was collected into polyethylene tubes and frozen at − 25 °C. Immediately after the decapitation, the brains were dissected from the skull, washed with saline and also frozen at − 25 °C. Serum and brain concentrations of caffeine and the tested antidepressants were assayed by the high performance liquid chromatography (HPLC) methods.

2.8. Determination of antidepressants in serum and brain homogenates

The brains were homogenized in distilled water (1:4, w/v) with a tissue homogenizer TH220 (Omni International, Inc., Warrenton, VA, USA). For the tested antidepressant drugs, the extraction from serum and brain homogenates were performed using the mixture of ethyl acetate:hexane (30:70, v/v). Paroxetine (40 or 200 ng/ml for fluoxetine and escitalopram, respectively) was used as an internal standard (IS). In order to isolate both drugs from biological material, appropriate amounts of IS were added to serum (200 µl) or brain homogenate (1 ml) containing these drugs and the samples were alkalized with 50 and 250 µl of 4 M NaOH, respectively. Then the samples were extracted with 5 ml of the extraction reagent by shaking for 20 min (IKA Vibrax VXR, Germany). After centrifugation at 3000 rpm for 20 min (Universal 32, Hettich, Germany), the organic layers were transferred to new tubes containing a 100 µl solution of 0.1 M H_2SO_4 and methanol (90:10, v/v), shaken for 0.5 h and then centrifuged for 15 min (3000 rpm). The organic layers were discarded and 50 µl aliquots of acidic solutions were injected into the HPLC system.

The HPLC system (Merck-Hitachi, Darmstadt, Germany) consisted of an L–7100 isocratic pump, an L–7200 autosampler, and a UV variable-wavelength K–2600 detector (Knauer, Berlin, Germany). D–7000 HSM software was used for data acquisition and processing. All analyses were performed on a 250 × 4.6 mm Supelcosil LC–CN column with a particle size of 5 µm (Sigma Aldrich, Germany) protected with a guard column with the same packing material. The mobile phase consisting of acetonitrile and 50 mM potassium dihydrogen phosphate was mixed at a ratio of 75:25 (v/v) and run at 1 ml/min. Chromatographic analysis was carried out at 21 °C and the analytical wavelength of 227 nm for fluoxetine, and 240 nm for escitalopram.

The calibration curves constructed by plotting the ratio of the peak heights of the studied drug to IS versus concentration of the drug were linear in the tested concentration ranges. No interfering peaks were observed in the chromatograms. The assays were reproducible with low intra- and inter-day variation (coefficient of variation < 10%). The extraction efficiencies of the analyzed compounds and internal standards ranged from 85 to 94%. Antidepressant concentrations were expressed in ng/ml of serum or ng/g of wet brain tissue.

2.9. Determination of caffeine in serum and brain homogenates

The brains were homogenized in phosphate buffer pH 7.2 (1:2, w/v) with a tissue homogenizer (Ultra Turrax T8, IKA, Germany). Caffeine extraction from serum (200 µl) and brain homogenate (300 µl) was performed by 6% perchloric acid protein precipitation. The mixture was vortexed for 30 s and centrifuged at 5000 rpm for 15 min. The supernatants were filtered through cellulose filter (nominal pore diameter 0.20 µm) and a 20 µl volume of each sample solution was injected into the HPLC system. The undertaken sample preparation method was the modification of the procedure described by Novitskayaa et al. (2013).

The HPLC system (Perkin Elmer Series 200, Shelton, CT, USA) consisted of a isocratic pump, a variable-wavelength UV/VIS detector, and an autosampler. All analyses were performed on a 250 × 4.6 mm Hypersil® column with a particle size of 5 µm (Thermo Electron Corporation, Waltham, MA, USA) protected with a guard column (4 × 4 mm) with the same packing material. The mobile phase consisting of water (brought to pH 4.0 with 1% formic acid):acetonitrile:methanol (80:8:14, v/v/v) was run at 1 ml/min. Chromatographic analysis was carried out at 21 °C and the analytical wavelength of 273 nm.

Calibration curves constructed based on the analysis of samples containing caffeine at concentrations covering the range of 1 to 24 µg/ml prepared for murine serum and brain homogenates were linear in the tested concentration ranges. No interference from the matrix at the retention time of caffeine was observed in the chromatograms. Caffeine concentrations were expressed in µg/ml of serum or ng/g of wet brain tissue.

2.10. The quantitative real-time PCR analysis (qRT-PCR)

At the appropriate time points after chronic administration of caffeine and a single dose of the antidepressant drugs given alone or simultaneously with caffeine, mice were decapitated; their brains were rapidly dissected and immersed in cooled (2–8 °C) saline. Cerebral cortex (Cx) was dissected on a cold plate, immediately frozen on dry ice and stored at − 80 °C until analysis.

qRT-PCR method was used to evaluate the expression of selected genes in the Cx. RNA was isolated from 30 mg of tissue using Syngen Tissue RNA Mini Kit (Syngen Biotech, Poland) and reverse transcription was performed by means of NG dART RT-PCR kit (EURx, Poland) according to the manufacturers' instructions. The relative expression of genes: *Slc6a15*, *Comt*, and *Adora1* (Mn00558415_m1, Mn00514377_m1, Mn01308023_m1, TaqMan Gene Expression Assays, Life Technologies, USA) was determined by qRT-PCR and the ΔΔCt method using hypoxanthine guanine phosphoribosyl transferase (HPRT) (Mn00446968_m1) as an endogenous control. The reaction was carried out in quadruplicate using the SmartChip Real-time PCR System (WaferGen Biosystems) and TaqMan Fast Universal PCR Master Mix (2 x) (Applied BioSystems, USA) according to manufacturer's instructions. The data quality screen based on amplification curves and Ct values was performed to remove any outlier data before ΔΔCt calculations and to determine fold change in mRNA levels. Statistical analysis was performed on RQ values ($RQ = 2 - \Delta\Delta Ct$).

2.11. Statistical analysis

The results obtained in the FST, the TST, locomotor activity and relative gene expression levels of *Slc6a15*, *Comt*, and *Adora1* were analyzed using two-way ANOVA followed by a Bonferroni's *post-hoc* test (when appropriate). The concentrations of caffeine and the tested antidepressants in serum and brains of mice were analyzed using a Student's *t*-test. The statistical analysis was carried out using GraphPad Prism for Windows version 4 (GraphPad Software, San Diego, CA, U.S.A.). All results are presented as the mean ± S.E.M. $p < 0.05$ was considered as statistically significant with 95% confidence.

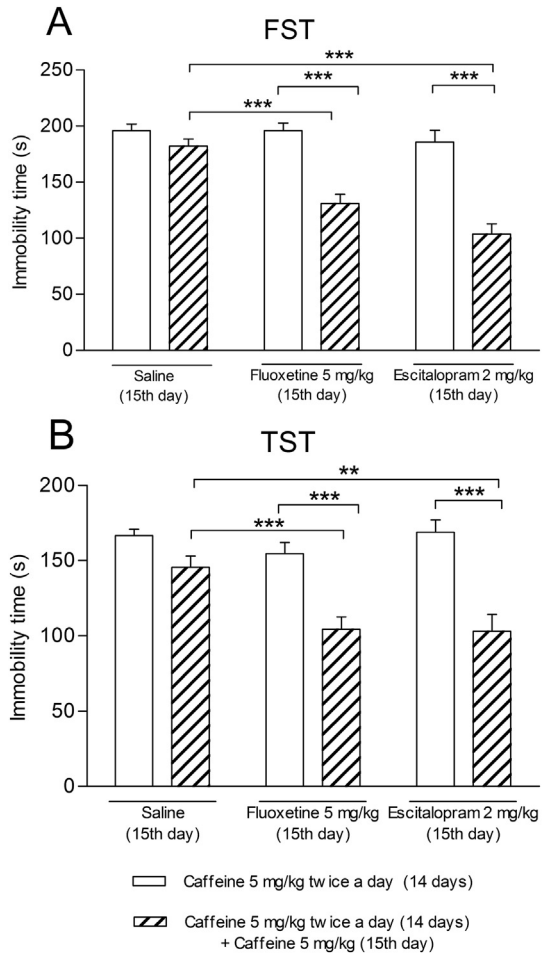

Fig. 1. The effects of a 14-day administration of caffeine and its withdrawal on day 15th on the activity of acute, per se inactive doses of fluoxetine and escitalopram in the forced swim test (FST) (A) and the tail suspension test (TST) (B) in mice. Caffeine was administered i.p. twice daily for 14 days and on day 15th it was withdrawn or administered 40 min before the tests, whereas saline or antidepressants (fluoxetine or escitalopram) were injected i.p. once, 60 min before the tests. **p < 0.01, ***p < 0.001 by the two-way ANOVA followed by a Bonferroni *post-hoc* test; values expressed as the mean + S.E.M., *n* = 9–10 per group.

3. Results

3.1. Forced swim test (FST)

The effects of a 14-day administration of caffeine and its withdrawal on day 15th on the activity of single, per se inactive doses of fluoxetine and escitalopram in the FST are shown in Fig. 1A. Withdrawal of caffeine on day 15th after a 14-day treatment did not induce significant changes in the total duration of immobility in the FST (compared to mice that after a 14-day treatment received caffeine also on day 15th). There were also no significant changes in the total duration of immobility between groups that after a 14-day treatment received on day 15th fluoxetine or escitalopram followed by caffeine withdrawal and group that received saline followed by caffeine withdrawal. However, when after a 14-day treatment caffeine was given on day 15th in addition to antidepressant drugs, a decrease in immobility time was observed in groups that received fluoxetine or escitalopram and caffeine, compared to mice that received saline and caffeine on this day ($p < 0.001$ for fluoxetine and escitalopram). Furthermore, co-administration of caffeine and fluoxetine ($p < 0.001$) or caffeine and escitalopram ($p < 0.01$) on 15th day decreased immobility time compared to the respective antidepressants given alone. A two-way ANOVA showed a significant effect of drug treatment [$F(2,53) = 8.695$, $p = 0.0005$], a significant effect of caffeine treatment [$F(1,53) = 68.59$, $p < 0.0001$] and a significant drug × caffeine treatment interaction [$F(2,53) = 8.695$, $p = 0.0005$].

3.2. Tail suspension test (TST)

The effects of a 14-day administration of caffeine and its withdrawal on day 15th on the activity of single, per se inactive doses of fluoxetine and escitalopram in the TST are shown in Fig. 1B. Withdrawal of caffeine on day 15th after a 14-day treatment did not significantly change the total duration of immobility in the TST (compared to mice that after a 14-day treatment received caffeine also on day 15th). Moreover, when after a 14-day treatment caffeine was withdrawn on 15th day, the total duration of immobility of mice that received fluoxetine or escitalopram did not differ from mice that received saline. However, when after a 14-day treatment caffeine was given on day 15th in addition to antidepressant drugs, a decrease in immobility time was observed compared to mice that received saline and caffeine on this day ($p < 0.001$ for fluoxetine; $p < 0.01$ for escitalopram). Moreover, co-administration of caffeine and fluoxetine ($p < 0.001$) or caffeine and escitalopram ($p < 0.01$) on 15th day decreased immobility time compared to the respective antidepressant given alone. A two-way ANOVA showed a significant effect of drug treatment [$F(2,53) = 5.846$, $p = 0.0051$], a significant effect of caffeine treatment [$F(1,53) = 47.29$, $p < 0.0001$] and a significant drug × caffeine treatment interaction [$F(2,53) = 3.918$, $p = 0.0259$].

3.3. Spontaneous locomotor activity

The effects of caffeine and drug treatment on the spontaneous locomotor activity of mice are shown in Table 1. There were no significant changes in the spontaneous locomotor activity between the examined groups. A two-way ANOVA showed no effect of drug treatment [$F(2,42) = 2.585$, $p = 0.0874$], no effect of caffeine treatment [$F(1,42) = 3.872$, $p = 0.0557$] and no drug × caffeine treatment interaction [$F(2,42) = 1.095$, $p = 0.3439$].

3.4. Pharmacokinetic studies

The effects of caffeine and drug treatment on serum and brain concentrations of the tested antidepressants given on the 15th day after chronic administration of caffeine in mice are shown in Table 2. In case of co-administration of caffeine and escitalopram on the 15th day after chronic administration of caffeine decreased escitalopram concentration in serum (*t*-test: $p = 0.0253$) was observed in comparison to the mice treated with escitalopram alone. The concentration of this drug in

Table 1
The effects of caffeine schedule and drug treatment on the spontaneous locomotor activity of mice.

Treatment (mg/kg)		Distance traveled (cm)
Twice daily for 14 days	15th day	
Caffeine 5	Saline	813.4 ± 179.8
Caffeine 5	Caffeine 5 + saline	777.8 ± 61.78
Caffeine 5	Fluoxetine 5	689.8 ± 90.04
Caffeine 5	Caffeine 5 + fluoxetine 5	696.6 ± 105.8
Caffeine 5	Escitalopram 2	736.8 ± 56.52
Caffeine 5	Caffeine 5 + escitalopram 2	979.4 ± 90.11

Caffeine was administered i.p. twice daily for 14 days and on day 15th it was withdrawn or administered 40 min before the test, whereas saline or antidepressants (fluoxetine or escitalopram) were injected i.p. once, 60 min before the test. Distance traveled was recorded between the 2nd and the 6th min of the test. Each experimental group consisted of 8 animals. Data are presented as the means ± S.E.M. The result was considered statistically significant if $p < 0.05$ (a two-way ANOVA followed by the Bonferroni's *post-hoc* test).

Table 2
The effects of caffeine schedule and drug treatment on the concentrations of antidepressants in mouse serum and brain.

Treatment (mg/kg)		Drug concentration	
Twice daily for 14 days	15th day	Serum (ng/ml)	Brain (ng/g)
Caffeine 5	Fluoxetine 5	323.4 ± 20.97	6521 ± 519.1
Caffeine 5	Caffeine 5 + fluoxetine 5	280.6 ± 12.82	6314 ± 448.3
Caffeine 5	Escitalopram 2	28.36 ± 3.745	185.4 ± 7.081
Caffeine 5	Caffeine 5 + escitalopram 2	16.99 ± 2.102*	147.6 ± 22.62

Caffeine was administered i.p. twice daily for 14 days and on day 15th it was withdrawn or administered 40 min before decapitation, whereas antidepressants (fluoxetine or escitalopram) were injected i.p. only once 60 min before decapitation. Each experimental group consisted of 10 animals. Results are presented as mean values ± S.E.M.

* $p < 0.05$ compared with the respective control group (Student's *t*-test).

the brain tissue was also lower, however, the difference was not significant (*t*-test: $p = 0.1139$). No statistically significant changes in concentrations of fluoxetine both in serum and brain homogenates were obtained in the group treated with caffeine and fluoxetine on the 15th day after chronic administration of caffeine *vs* the group receiving only the antidepressant drug on this day (*t*-test: $p = 0.0981$ and $p = 0.7663$, respectively).

The effect of tested drugs on serum and brain concentrations of caffeine given on the 15th day after chronic administration of caffeine in mice is show in Table 3. In case of joint administration of caffeine and escitalopram on the 15th day after chronic administration of caffeine a significant increase in caffeine concentration in serum (*t*-test: $p = 0.0066$) but not in the brain tissue (*t*-test: $p = 0.2262$) was observed. No statistically significant changes in the concentrations of caffeine both in serum and brain homogenates were obtained in the group treated with caffeine and fluoxetine on the 15th day after chronic administration of caffeine *vs* the group treated with fluoxetine alone (*t*-test: $p = 0.3711$ and $p = 0.2597$, respectively).

3.5. Gene expression studies

The effects of caffeine and drug treatment on the mRNA level of *Comt* in the Cx are shown in Fig. 2A. There were no significant changes in *Comt* mRNA level in the Cx between the examined groups. A two-way ANOVA showed no effect of caffeine treatment [$F(1,24) = 0.01455$, $p = 0.9050$], no effect of drug treatment [$F(2,24) = 0.2476$, $p = 0.7826$] and no caffeine × drug treatment interaction [$F(2,24) = 1.323$, $p = 0.2851$].

The effects of caffeine and drug treatment on the mRNA level of *Slc6a15* in the Cx are shown in Fig. 2B. Administration of fluoxetine ($p < 0.001$) or escitalopram ($p < 0.01$) on day 15th together with caffeine induced a decrease in *Slc6a15* mRNA level in the Cx, compared to mice that received saline together with caffeine on this day.

Table 3
The effects of caffeine schedule and drug treatment on the concentrations of caffeine in mouse serum and brain.

Treatment (mg/kg)		Caffeine concentration	
Twice daily for 14 days	15th day	Serum (μg/ml)	Brain (ng/g)
Caffeine 5	Caffeine 5 + saline	3.030 ± 0.3710	539.6 ± 90.47
Caffeine 5	Caffeine 5 + fluoxetine 5	3.393 ± 0.2050	433.7 ± 29.05
Caffeine 5	Caffeine 5 + escitalopram 2	4.123 ± 0.1549*	419.5 ± 45.82

Caffeine was administered i.p. twice daily for 14 days and on day 15th it was administered 40 min before decapitation, whereas saline or antidepressants (fluoxetine or escitalopram) were injected i.p. only once 60 min before decaptitation. Each experimental group consisted of 10 animals. Results are presented as mean values ± S.E.M.

* $p < 0.01$ compared with the respective control group (Student's *t*-test).

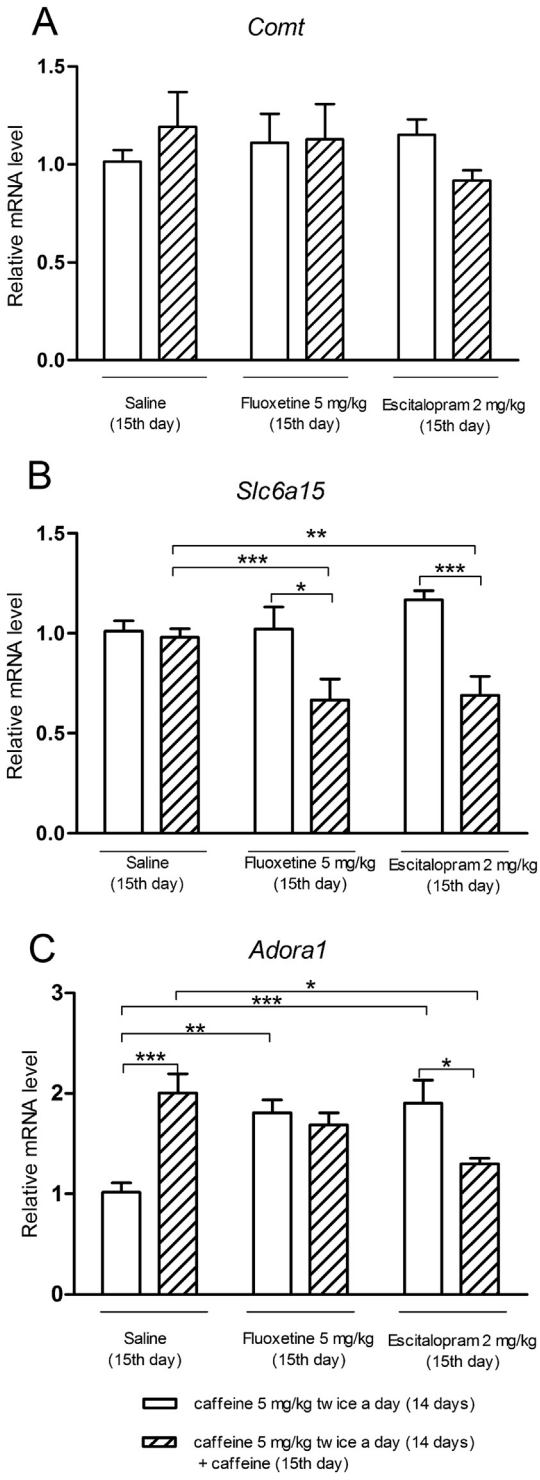

Fig. 2. The effects of caffeine schedule and drug treatment on the mRNA level of catechol-*O*-methyltransferase (*Comt*) (A), solute carrier family 6 member 15 (*Slc6a15*) (B) and adenosine A_1 receptor (*Adora1*) (C) genes in the cerebral cortex. Caffeine was administered i.p. twice daily for 14 days and on day 15th it was withdrawn or administered 40 min before the tests, whereas saline or antidepressants (fluoxetine or escitalopram) were injected i.p. once, 60 min before the tests. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by the two-way ANOVA followed by a Bonferroni *post-hoc* test; values expressed as the mean + S.E.M., $n = 9$–10 per group.

Moreover, administration of fluoxetine ($p < 0.05$) or escitalopram ($p < 0.001$) on day 15th together with caffeine induced a decrease in *Slc6a15* mRNA level in the Cx, compared to mice that received fluoxetine or escitalopram followed by caffeine withdrawal. A two-way ANOVA showed a significant effect of caffeine treatment [$F(1,24) = 19.12$, $p = 0.0002$], no effect of drug treatment [$F(2,24) = 1.784$, $p = 0.1896$] and a significant caffeine × drug treatment interaction [$F(2,24) = 4.085$, $p = 0.0297$].

The effects of caffeine and drug treatment on the mRNA level of *Adora1* in the Cx are shown in Fig. 2C. Caffeine withdrawal on day 15th following a 14-day treatment induced a decrease in *Adora1* mRNA level in the Cx, compared to group that after a 14-day treatment received caffeine also on the 15th day ($p < 0.001$). Administration of fluoxetine ($p < 0.01$) or escitalopram ($p < 0.001$) in mice in whom caffeine was withdrawn caused an increase in *Adora1* mRNA level in the Cx, compared to mice that received saline followed by caffeine withdrawal. Moreover, administration of escitalopram, but not fluoxetine, on day 15th together with caffeine caused a decrease in *Adora1* mRNA level in the Cx, compared to mice that received saline together with caffeine on this day ($p < 0.05$), as well as compared to mice that received escitalopram followed by caffeine withdrawal ($p < 0.05$). A two-way ANOVA demonstrated no effect of caffeine treatment [$F(1,24) = 0.5126$, $p = 0.4809$], no effect of drug treatment [$F(2,24) = 1.283$, $p = 0.2956$], but a significant caffeine × drug treatment interaction [$F(2,24) = 14.97$, $p < 0.0001$].

4. Discussion

Adenosine is commonly known as a neuromodulator in the CNS which inhibits synaptic activity and neurotransmitter release (Jarvis and Williams, 1990). Under physiological conditions adenosine exerts its central effects via activation of the A_1 and A_{2A} adenosine receptors. Both receptors are the preferred targets of methylxanthines, including caffeine (Barone and Roberts, 1996; Fredholm et al., 1999). Preclinical and clinical data have indicated a relationship between depressive-like behavior and the purinergic signaling (El Yacoubi et al., 2001; El Yacoubi et al., 2003; Szopa et al., 2016; Blardi et al., 2005; Berk et al., 2001; Elgün et al., 1999). Caffeine possesses antidepressant-like activity in animal tests and models of depression (El Yacoubi et al., 2003; Szopa et al., 2016; Sezer et al., 2007). It has been reported that an acute administration of caffeine produced dose-dependent changes in the depressive-like behavior in the FST in mice (Enríquez-Castillo et al., 2008; Szopa et al., 2016; Vieira et al., 2008; El Yacoubi et al., 2003). Moreover, our previous studies showed that caffeine (a non-selective adenosine receptors antagonist) enhances the antidepressant-like activity of antidepressant drugs with different mechanisms of action (i.a., imipramine, desipramine, fluoxetine, escitalopram, paroxetine, agomelatine, milnacipran, bupropion, and moclobemide) (Poleszak et al., 2015; Szopa et al., 2016; Poleszak et al., 2016). However, there is lack of information about the effects of a chronic administration of caffeine on the treatment with SSRIs. Some studies revealed a dose-dependent up-regulation of A_1 and A_{2A} receptors in the mouse brain, suggesting an adaptive effect of caffeine intake (Nikodijević et al., 1993), notwithstanding the potency of this xanthine as an antagonist of the endogenous adenosine is not modified by tolerance (Holtzman et al., 1991).

Here we evaluated the impact of low dose of caffeine administered chronically (twice daily for 14 days) and its withdrawal on the antidepressant-like activity of single, per se inactive doses of SSRIs, fluoxetine and escitalopram, in two basic tests (i.e., the FST, TST) used for antidepressant activity assessment. Since no alterations in the spontaneous locomotor activity were found, the results indicate antidepressant-like activity of low, ineffective doses of SSRIs administered together with caffeine following a long-term caffeine intake. Caffeine through antagonism of the adenosine A_1 receptors located on the 5-HT neurons (Mössner et al., 2000; Regenold and Illes, 1990) blocks the effects of endogenous adenosine and increases 5-HT, NA (Fredholm et al., 1999; Daly and Fredholm, 1998), and DA (Kull et al., 2000; Salmi et al., 2005; Corvol et al., 2001) transduction in the brain. Activation of NA neurons and its interaction with the central dopaminergic system have been reported in several studies with caffeine (Ferré, 2008; Fisone et al., 2004; Nehlig et al., 1992). Therefore, the shortening of the immobility time after the joint administration of caffeine and SSRIs, fluoxetine or escitalopram, may be a consequence of the concomitant enhancement of monoamine (5-HT, NA and DA) transmission.

Bilateral interaction between caffeine and other CNS-active substances in the pharmacodynamic and pharmacokinetic phase are feasible and may affect depression pharmacotherapy (Donovan and DeVane, 2001; Kot et al., 2007). The literature data emphasize the role of CYP1A2, the main enzyme responsible for the metabolism of caffeine (Daniel et al., 2003), in the metabolism of antidepressants (e.g., imipramine, mianserin, amitriptyline, duloxetine, bupropion or fluvoxamine) (Jefferson et al., 2005; Lin and Lu, 1998; Pelkonen et al., 1998; Lantz et al., 2003). Therefore, we carried out a pharmacokinetic analysis aimed at determination of the concentrations of the tested antidepressants and caffeine in serum and brain after their combined administration, estimating the nature of the agent-agent interactions. We observed that caffeine did not affect the concentration of fluoxetine, either in serum or in brain homogenates. Likewise, there were no statistically significant changes in the concentration of caffeine in the tested biological material in the group receiving caffeine and fluoxetine, which suggests that a caffeine/fluoxetine interaction presumably is pharmacodynamic in nature. The interplay between caffeine and escitalopram demonstrated in the course of our study is not entirely clear. In case of concurrent administration of escitalopram and caffeine a statistically significant reduction in the concentration of escitalopram in the serum of animals, with no statistically significant changes in the brain tissue were observed. In turn, in this drug combination a considerable increase in the level of caffeine in the serum, but no changes in brain homogenates were observed. It is possible that the observed alterations were associated with the modifications in biotransformation of escitalopram and/or caffeine, since both caffeine and escitalopram are metabolized by the isoenzymes of cytochrome P450 (Kuo et al., 2013; Begas et al., 2007). It is puzzling that the observed changes in the levels of escitalopram and caffeine in serum did not contribute to the substantial changes in their concentrations in the brain tissue. Lack of such relationship may be due to the retardation in the transport through the blood-brain barrier of both compounds (Burke and Preskorn, 2004). The recorded changes in the concentrations of SSRIs and caffeine in the tested biological material are ambiguous and may be associated with a dose-dependent metabolism of caffeine, i.e., the rate of elimination decreases with an increasing caffeine dose (Denaro et al., 1990). Therefore, the application in our study of caffeine at a low dose (5 mg/kg) could result in the drug-drug interaction that was not very pronounced (Kale and Addepalli, 2014).

To elucidate possible mechanisms underlying the effects observed following caffeine and antidepressant treatment, we next measured relative gene expression of the selected genes that have been implicated in the pathophysiology and/or pharmacotherapy of depression (Blazquez et al., 2012; O'Leary et al., 2014; Choi et al., 2016; Santarelli et al., 2016; Serchov et al., 2015). *Comt* is an intracellular enzyme localized in postsynaptic membrane of neurons (Ulmanen et al., 1997). It is responsible for degradation of DA, adrenaline, and NA (Eisenhofer et al., 2004). Several studies have reported that met/met genotype of the Val(108)Met polymorphism in *Comt* is associated with a better response to a variety of antidepressants, including fluoxetine and other SSRIs (O'Leary et al., 2014). We found no significant differences in the *Comt* mRNA level in animals treated for 14 days with caffeine and on day 15th day with antidepressants simultaneously with or without caffeine. It has been demonstrated that fluoxetine downregulates the expression of *Comt* at mRNA level, this effect was however observed after chronic (a 21-day) treatment (Fatemi and Folsom, 2007).

Therefore, a single, low doses of the tested antidepressant drugs may not be sufficient to modulate *Comt* expression.

Genome-wide association study has revealed *Slc6a15* as a novel susceptibility gene for depression. Risk allele carrier status in humans and chronic stress in mice were associated with a downregulation of the expression of this gene in the hippocampus. The same polymorphisms showed associations with alterations in hippocampal volume and neuronal integrity (Kohli et al., 2011). Here, antidepressant-like activity of low doses of SSRIs administered together with caffeine on day 15th following 14 days of treatment with caffeine was associated with decreased mRNA level of *Slc6a15* in the Cx. It was shown that chronic (a 14-day) treatment with low (3 mg/kg) or high (10 mg/kg) dose of fluoxetine caused an up-regulation of *Slc6a15* at mRNA level in the hippocampus (Hägglund et al., 2013), suggesting the role of *Slc6a15* in the mode of action of antidepressant drugs acting on the serotonergic system. Our study further supports the involvement of *Slc6a15* in the mechanisms of action of antidepressants targeting this system. It is plausible that a down-regulation of *Slc6a15* occurs after a single dose of SSRIs (as shown in the present study), whereas up-regulation after chronic treatment (Hägglund et al., 2013). Furthermore, although a withdrawal of caffeine on the 15th day did not influence *Slc6a15* mRNA level, a decrease in *Slc6a15* mRNA level was observed when after a 14-day caffeine administration it was given together with antidepressants on day 15th, compared to antidepressants given on this day alone.

Slc615 transports neutral amino acids such as proline, which can be used in the biosynthesis of the main excitatory neurotransmitter, glutamate. Glutamate, besides monoamine neurotransmitters, has been established to be involved in the pathophysiology of depression (Pilc et al., 2013). *Slc6a15* expression was shown to correlate with proline and glutamate content of the hippocampus (Santarelli et al., 2015), moreover, *Slc6a15* knockout animals displayed lower levels of depressive-like behavior following chronic stress, while *Slc6a15* overexpression animals displayed an increase in depressive-like behavior, together with increased GluR1 subunit of the glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mRNA expression in subregions of the hippocampus (Santarelli et al., 2016). Therefore, our findings indicate a possible role of *Slc6a15* in mediating antidepressant-like effects of fluoxetine and escitalopram administered simultaneously with caffeine following chronic caffeine intake period.

Furthermore, we assessed *Adora1*, which encodes A_1 receptor, relative gene expression. We found that withdrawal of caffeine (A_1 receptors antagonist) decreased *Adora1* mRNA level. It is plausible that administration of low dose of caffeine following its chronic treatment induces an increase in *Adora1* mRNA, compared to mice in which caffeine was withdrawn on 15th day, as a compensatory mechanism. It has been shown using a transgenic mouse model with conditioned up-regulation of A_1 receptors selectively in forebrain that increasing A_1 expression evokes resilience against depressive-like behavior in the FST and TST as well as antidepressant effects in chronic stress model (Serchov et al., 2015). Changes in *Adora1* expression were in the present study not parallel to behavioral antidepressant-like effect of low, ineffective doses of antidepressants administered simultaneously with caffeine on day 15th after a 14-day caffeine treatment. However, administration of the tested SSRIs in animals in whom caffeine was withdrawn caused an increase in *Adora1* mRNA expression. In contrast, administration of escitalopram simultaneously with caffeine on day 15th caused a decrease in this gene expression, compared to mice that received the antidepressant and in whom caffeine was withdrawn. This effect was associated with decreased immobility time in two behavioral tests that were used. Therefore, an interplay between the schedule of caffeine intake (chronic intake followed by withdrawal or not) and antidepressant drug treatment, may have led to differential regulation of *Adora1* expression, which may have a consequence for A_1 receptor function. In addition to inhibitory A_1 receptors, caffeine antagonizes excitatory A_{2A} receptors. Both receptors are widely distributed in brain regions such as cerebral cortex and are highly localized to synaptic regions, where they modulate the release of neurotransmitters: monoamine neurotransmitters and amino acid neurotransmitters (glutamate, GABA) (Sheth et al., 2014). A_{2A} receptors were found to be involved in antidepressant-like effect (Yamada et al., 2013). Therefore, chronic treatment with caffeine and subsequent administration of antidepressant drugs may cause changes not only in A_1 receptors but also in A_{2A} receptors expression/function, which may lead to regulation of neurotransmitters release.

5. Conclusions

Our findings show antidepressant-like activity of single, per se ineffective doses of fluoxetine and escitalopram in two basic tests (i.e., the FST, TST) used for antidepressant-like activity assessment, in mice in which caffeine was given in addition to antidepressants (fluoxetine, escitalopram) following a period of chronic (a 14-day) treatment with caffeine, in contrast to mice, in whom caffeine was withdrawn after a period of its chronic administration. Our findings reveal a possible role of *Slc6a15* in mediating this effect. Our results suggest that a schedule of caffeine intake (chronic intake followed by withdrawal or not) may affect the activity of commonly used antidepressants belonging to the SSRI group. Moreover, the schedule of caffeine intake and a single dose of studied antidepressants can modulate *Adora1* expression. Given the physiological function of *Slc6a15* and *Adora1*, these results suggest that concomitant administration of caffeine and antidepressants can have an impact on systems implicated in depression pathophysiology and treatment, i.e., glutamate, adenosine and monoamine systems.

Ethical approval

All procedures were conducted in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and Polish legislation acts concerning animal experimentations. The experimental procedures and protocols were approved by the First Local Ethics Committee at the Medical University of Lublin.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

This study was supported by Funds for Statutory Activity of Medical University of Lublin, Poland (MNmb 18). The authors wish to thank Chair and Department of Hygiene of Medical University in Lublin for access to an animal activity meter Opto-Varimex-4 Auto-Track and Karol Iwaniak, Karol Rojek and Karolina Bogatko for their excellent technical assistance.

References

Barone, J.J., Roberts, H.R., 1996. Caffeine consumption. Food Chem. Toxicol. 34, 119–129.
Begas, E., Kouvaras, E., Tsakalof, A., Papakosta, S., Asprodini, E.K., 2007. *In vivo* evaluation of CYP1A2, CYP2A6, NAT-2 and xanthine oxidase activities in a Greek population sample by the RP-HPLC monitoring of caffeine metabolic ratios. Biomed. Chromatogr. 21, 190–200.
Benowitz, N.L., 1990. Clinical pharmacology of caffeine. Annu. Rev. Med. 41, 277–288.
Berk, M., Plein, H., Ferreira, D., Jersky, B., 2001. Blunted adenosine A2a receptor function in platelets in patients with major depression. Eur. Neuropsychopharmacol. 11, 183–186.
Blardi, P., de, L.A., Urso, R., Auteri, A., Dell'Erba, A., Bossini, L., Castrogiovanni, P., 2005. Activity of citalopram on adenosine and serotonin circulating levels in depressed patients. J. Clin. Psychopharmacol. 25, 262–266.
Blazquez, A., Mas, S., Plana, M.T., Lafuente, A., Lázaro, L., 2012. Fluoxetine pharmacogenetics in child and adult populations. Eur. Child Adolesc. Psychiatry 21, 599–610.
Burke, M.J., Preskorn, S.H., 2004. Therapeutic drug monitoring of antidepressants. In: Preskorn, S.H., Feigher, J.P., Stanga, C.Y., Ross, R. (Eds.), Antidepressants: Past, Present and Future. Springer-Verlag, Heidelberg, pp. 87–117.
Choi, S., Han, K.M., Kang, J., Won, E., Chang, H.S., Tae, W.S., Son, K.R., Kim, S.J., Lee,

M.S., Ham, B.J., 2016. Effects of a polymorphism of the neuronal amino acid transporter *SLC6A15* gene on structural integrity of white matter tracts in major depressive disorder. PLoS One 11, e0164301.
Corvol, J.C., Studler, J.M., Schonn, J.S., Girault, J.A., Herve, D., 2001. $G\alpha_{olf}$ is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. J. Neurochem. 76, 1585–1588.
Daly, J.W., Fredholm, B.B., 1998. Caffeine – an atypical drug of dependence. Drug Alcohol Depend. 51, 199–206.
Daniel, W.A., Kot, M., Wójcikowski, J., 2003. Effects of classic and newer antidepressants on the oxidation pathways of caffeine in rat liver. In vitro study. Pol. J. Pharmacol. 55, 1045–1053.
Denaro, C.P., Brown, C.R., Wilson, M., Jacob III, P., Benowitz, N.L., 1990. Dose-dependency of caffeine metabolism with repeated dosing. Clin. Pharmacol. Ther. 48, 277–285.
Donovan, J.L., DeVane, C.L., 2001. A primer on caffeine pharmacology and its drug interactions in clinical psychopharmacology. Psychopharmacol. Bull. 35, 30–48.
Eisenhofer, G., Kopin, I.J., Goldstein, D.S., 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol. Rev. 56, 331–349.
El Yacoubi, M., Ledent, C., Parmentier, M., Bertorelli, R., Ongini, E., Costentin, J., Vaugeois, J.M., 2001. Adenosine A_{2A} receptor antagonists are potential antidepressants: evidence based on pharmacology and A_{2A} receptor knockout mice. Br. J. Pharmacol. 134, 68–77.
El Yacoubi, M., Costentin, J., Vaugeois, J.M., 2003. Adenosine A_{2A} receptors and depression. Neurology 61, S82–S87.
Elgün, S., Keskinege, A., Kumbasar, H., 1999. Dipeptidyl peptidase IV and adenosine deaminase activity. Decrease in depression. Psychoneuroendocrinology 24, 823–832.
Enríquez-Castillo, A., Alamilla, J., Barral, J., Gourbière, S., Flores-Serrano, A.G., Góngora-Alfaro, J.L., Pineda, J.C., 2008. Differential effects of caffeine on the antidepressant-like effect of amitriptyline in female rat subpopulations with low and high immobility in the forced swimming test. Physiol. Behav. 94, 501–509.
Fatemi, S.H., Folsom, T.D., 2007. Catechol-*O*-methyltransferase gene regulation in rat frontal cortex. Mol. Psychiatry 12, 322–323.
Ferré, S., 2008. An update on the mechanisms of the psychostimulant effects of caffeine. J. Neurochem. 105, 1067–1079.
Fisone, G., Borgkvist, A., Usiello, A., 2004. Caffeine as a psychomotor stimulant: mechanism of action. Cell. Mol. Life Sci. 61, 857–872.
Fredholm, B.B., 1995. Astra award lecture. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol. Toxicol. 76, 93–101.
Fredholm, B.B., Battig, K., Holmén, J., Nehlig, A., Zvartau, E.E., 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51, 83–133.
Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Klotz, K.N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.
Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Linden, J., Muller, C.E., 2011. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. Pharmacol. Rev. 63, 1–34.
Fredholm, B.B., Yang, J., Wang, Y., 2016. Low, but not high, dose caffeine is a readily available probe for adenosine actions. Mol. Asp. Med. 55, 20–25.
Garcia-Blanco, T., Davalos, A., Visioli, F., 2016. Tea, cocoa, coffee, and affective disorders: vicious or virtuous cycle? J. Affect. Disord. http://dx.doi.org/10.1016/j.jad.2016.11.033.
Gasior, M., Borowicz, K., Kleinrok, Z., Czuczwar, S.J., 1996. Chronic caffeine and the anticonvulsant potency of antiepileptic drugs against maximal electroshock. Pharmacol. Biochem. Behav. 54, 639–644.
Hägglund, M.G., Hellsten, S.V., Bagchi, S., Ljungdahl, A., Nilsson, V.C., Winnergren, S., Stephansson, O., Rumaks, J., Svirskis, S., Klusa, V., Schiöth, H.B., Fredriksson, R., 2013. Characterization of the transporterB^0AT3 (*Slc6a17*) in the rodent central nervous system. BMC Neurosci. 14, 54.
Heckman, M.A., Weil, J., Gonzalez De Mejia, E., 2010. Caffeine (1,3,7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. J. Food Sci. 75, R77–R87.
Holtzman, S.G., Mante, S., Minneman, K.P., 1991. Role of adenosine receptors in caffeine tolerance. J. Pharmacol. Exp. Ther. 256, 62–68.
Jarvis, M., Williams, M., 1990. Adenosine in the central nervous system. In: Williams, M. (Ed.), Adenosine and Adenosine Receptors. Humana Press Inc., New Jersey, pp. 423–460.
Jefferson, J.W., Pradko, J.F., Muir, K.T., 2005. Bupropion for major depressive disorder: pharmacokinetic and formulation considerations. Clin. Ther. 27, 1685–1695.
Kale, P.P., Addepalli, V., 2014. Augmentation of antidepressant effects of duloxetine and bupropion by caffeine in mice. Pharmacol. Biochem. Behav. 124, 238–244.
Kale, P.P., Addepalli, V., Bafna, P.A., Prabhavalkar, K.S., 2010. Caffeine-induced augmentation of antidepressant therapy. J. Exp. Clin. Med. 2, 282–286.
Kohli, M.A., Lucae, S., Saemann, P.G., Schmidt, M.V., Demirkan, A., Hek, K., Czamara, D., Alexander, M., Salyakina, D., Ripke, S., Hoehn, D., Specht, M., Menke, A., Hennings, J., Heck, A., Wolf, C., Ising, M., Schreiber, S., Czisch, M., Muller, M.B., Uhr, M., Bettecken, T., Becker, A., Schramm, J., Rietschel, M., Maier, W., Bradley, B., Ressler, K.J., Nothen, M.M., Cichon, S., Craig, I.W., Breen, G., Lewis, C.M., Hofman, A., Tiemeier, H., van Duijn, C.M., Holsboer, F., Muller-Myhsok, B., Binder, E.B., 2011. The neuronal transporter gene *SLC6A15* confers risk to major depression. Neuron 70, 252–265.
Kot, M., Wójcikowski, J., Daniel, W.A., 2007. Caffeine metabolism during prolonged treatment of rats with antidepressant drugs. Pharmacol. Rep. 59, 727–733.
Kull, B., Svenningsson, P., Fredholm, B.B., 2000. Adenosine A_{2A} receptors are colocalized with and activate G_{olf} in rat striatum. Mol. Pharmacol. 58, 771–777.
Kuo, H.W., Liu, S.C., Tsou, H.H., Liu, S.W., Lin, K.M., Lu, S.C., Hsiao, M.C., Hsiao, C.F., Liu, C.Y., Chen, C.H., Lu, M.L., Shen, W.W., Tang, H.S., Liu, S.I., Chang, L.H., Wu, H.Y., Chang, Y.S., Yeh, T.K., Chen, A.C., Liu, Y.L., 2013. CYP1A2 genetic polymorphisms are associated with early antidepressant escitalopram metabolism and adverse reactions. Pharmacogenomics 14, 1191–1201.
Lantz, R.J., Gillespie, T.A., Rash, T.J., Kuo, F., Skinner, M., Kuan, H.Y., Knadler, M.P., 2003. Metabolism, excretion, and pharmacokinetics of duloxetine in healthy human subjects. Drug Metab. Dispos. 31, 1142–1150.
Leibenluft, E., Fiero, P.L., Bartko, J.J., Moul, D.E., Rosenthal, N.E., 1993. Depressive symptoms and the self-reported use of alcohol, caffeine, and carbohydrates in normal volunteers and four groups of psychiatric outpatients. Am. J. Psychiatry 150, 294–301.
Lin, J.H., Lu, A.Y., 1998. Inhibition and induction of cytochrome P450 and the clinical implications. Clin. Pharmacokinet. 35, 361–390.
Liu, Q.S., Deng, R., Fan, Y., Li, K., Meng, F., Li, X., Liu, R., 2017. Low dose of caffeine enhances the efficacy of antidepressants in major depressive disorder and the underlying neural substrates. Mol. Nutr. Food Res. 61, 10.
Lucas, M., Mirzaei, F., Pan, A., Okereke, O.I., Willett, W.C., O'Reilly, E.J., Koenen, K., Ascherio, A., 2011. Coffee, caffeine, and risk of depression among women. Arch. Intern. Med. 171, 1571–1578.
Lucas, M., O'Reilly, E.J., Pan, A., Mirzaei, F., Willett, W.C., Okereke, O.I., Ascherio, A., 2014. Coffee, caffeine, and risk of completed suicide: results from three prospective cohorts of American adults. World J. Biol. Psychiatry 15, 377–386.
Mössner, R., Albert, D., Persico, A.M., Hennig, T., Bengel, D., Holtmann, B., Schmitt, A., Keller, F., Simantov, R., Murphy, D., Seif, I., Deckert, J., Lesch, K.P., 2000. Differential regulation of adenosine A_1 and A_{2A} receptors in serotonin transporter and monoamine oxidase A-deficient mice. Eur. Neuropsychopharmacol. 10, 489–493.
Nehlig, A., 1999. Are we dependent upon coffee and caffeine? A review on human and animal data. Neurosci. Biobehav. Rev. 23, 563–576.
Nehlig, A., Debry, G., 1994. Potential teratogenic and neurodevelopmental consequences of coffee and caffeine exposure: a review on human and animal data. Neurotoxicol. Teratol. 16, 531–543.
Nehlig, A., Daval, J.L., Debry, G., 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res. Brain Res. Rev. 17, 139–170.
Nikodijević, O., Jacobson, K.A., Daly, J.W., 1993. Locomotor activity in mice during chronic treatment with caffeine and withdrawal. Pharmacol. Biochem. Behav. 44, 199–216.
Novitskayaa, Y.G., Litvina, A.A., Zherdeva, V.P., Blynskayaa, E.V., Kondabov, K.S., 2013. Assay of caffeine and its metabolites in the rat blood plasma using HPLC as a method for determination of metabolic ratios. Moc. Univ. Chem. Bul. 68, 47–50.
Nutt, D.J., 2006. The role of dopamine and norepinephrine in depression and antidepressant treatment. J. Clin. Psychiatry 67 (Suppl. 6), 3–8.
O'Leary, O.F., O'Brien, F.E., O'Connor, R.M., Cryan, J.F., 2014. Drugs, genes and the blues: pharmacogenetics of the antidepressant response from mouse to man. Pharmacol. Biochem. Behav. 123, 55–76.
Pelkonen, O., Mäenpää, J., Taavitsainen, P., Rautio, A., Raunio, H., 1998. Inhibition and induction of human cytochrome P450 (CYP) enzymes. Xenobiotica 28, 1203–1253.
Pilc, A., Wierońska, J.M., Skolnick, P., 2013. Glutamate-based antidepressants: preclinical psychopharmacology. Biol. Psychiatry 73, 1125–1132.
Poleszak, E., Szopa, A., Wyska, E., Wośko, S., Serefko, A., Wlaź, A., Pieróg, M., Wróbel, A., Wlaź, P., 2015. The influence of caffeine on the activity of moclobemide, venlafaxine, bupropion and milnacipran in the forced swim test in mice. Life Sci. 136, 13–18.
Poleszak, E., Szopa, A., Wyska, E., Kukuła-Koch, W., Serefko, A., Wośko, S., Bogatko, K., Wróbel, A., Wlaź, P., 2016. Caffeine augments the antidepressant-like activity of mianserin and agomelatine in forced swim and tail suspension tests in mice. Pharmacol. Rep. 68, 56–61.
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.
Pramod, A.B., Foster, J., Carvelli, L., Henry, L.K., 2013. SLC6 transporters: structure, function, regulation, disease association and therapeutics. Mol. Asp. Med. 34, 197–219.
Regenold, J.T., Illes, P., 1990. Inhibitory adenosine A_1-receptors on rat locus coeruleus neurones. An intracellular electrophysiological study. Naunyn Schmiedeberg's Arch. Pharmacol. 341, 225–231.
Rihs, M., Muller, C., Baumann, P., 1996. Caffeine consumption in hospitalized psychiatric patients. Eur. Arch. Psychiatry Clin. Neurosci. 246, 83–92.
Robles-Molina, E., Millan, D., Hong, E., Huang, F., Villafana, S., 2012. Increased antidepressant-like effect of desipramine combined with central stimulants (caffeine and amphetamine) in mice. Centr. Eur. J. Biol. 7, 391–396.
Ruusunen, A., Lehto, S.M., Tolmunen, T., Mursu, J., Kaplan, G.A., Voutilainen, S., 2010. Coffee, tea and caffeine intake and the risk of severe depression in middle-aged Finnish men: the Kuopio Ischaemic heart disease risk factor study. Public Health Nutr. 13, 1215–1220.
Salmi, P., Chergui, K., Fredholm, B.B., 2005. Adenosine-dopamine interactions revealed in knockout mice. J. Mol. Neurosci. 26, 239–244.
Santarelli, S., Namendorf, C., Anderzhanova, E., Gerlach, T., Bedenk, B., Kaltwasser, S., Wagner, K., Labermaier, C., Reichel, J., Drgonova, J., Czisch, M., Uhr, M., Schmidt, M.V., 2015. The amino acid transporter SLC6A15 is a regulator of hippocampal neurochemistry and behavior. J. Psychiatr. Res. 68, 261–269.
Santarelli, S., Wagner, K.V., Labermaier, C., Uribe, A., Dournes, C., Balsevich, G., Hartmann, J., Masana, M., Holsboer, F., Chen, A., Muller, M.B., Schmidt, M.V., 2016. SLC6A15, a novel stress vulnerability candidate, modulates anxiety and depressive-like behavior: involvement of the glutamatergic system. Stress 19, 83–90.
Serchov, T., Clement, H.W., Schwarz, M.K., Iasevoli, F., Tosh, D.K., Idzko, M., Jacobson, K.A., de Bartolomeis, A., Normann, C., Biber, K., van Calker, D., 2015. Increased

signaling via adenosine A_1 receptors, sleep deprivation, imipramine, and ketamine inhibit depressive-like behavior via induction of Homer1a. Neuron 87, 549–562.
Serefko, A., Szopa, A., Wlaź, A., Wośko, S., Wlaź, P., Poleszak, E., 2016. Synergistic antidepressant-like effect of the joint administration of caffeine and NMDA receptor ligands in the forced swim test in mice. J. Neural Transm. 123, 463–472.
Sezer, G., Sezer, Z., Tekol, Y., 2007. Role of adenosine and adenosine A_{2A} receptors in the antidepressant effect of amitriptyline. Eur. Neuropsychopharmacol. 17, 364.
Sheth, S., Brito, R., Mukherjea, D., Rybak, L.P., Ramkumar, V., 2014. Adenosine receptors: expression, function and regulation. Int. J. Mol. Sci. 15, 2024–2052.
Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berlin) 85, 367–370.
Szopa, A., Poleszak, E., Wyska, E., Serefko, A., Wośko, S., Wlaź, A., Pieróg, M., Wróbel, A., Wlaź, P., 2016. Caffeine enhances the antidepressant-like activity of common antidepressant drugs in the forced swim test in mice. Naunyn Schmiedeberg's Arch. Pharmacol. 389, 211–221.
Ulmanen, I., Peranen, J., Tenhunen, J., Tilgmann, C., Karhunen, T., Panula, P., Bernasconi, L., Aubry, J.P., Lundstrom, K., 1997. Expression and intracellular localization of catechol *O*-methyltransferase in transfected mammalian cells. Eur. J. Biochem. 243, 452–459.
Vaccarino, A.L., Evans, K.R., Kalali, A.H., Kennedy, S.H., Engelhardt, N., Frey, B.N., Greist, J.H., Kobak, K.A., Lam, R.W., MacQueen, G., Milev, R., Placenza, F.M., Ravindran, A.V., Sheehan, D.V., Sills, T., Williams, J.B., 2016. The Depression Inventory Development Workgroup: a collaborative, empirically driven initiative to develop a new assessment tool for major depressive disorder. Innov. Clin. Neurosci. 13, 20–31.
Vieira, C., De Lima, T.C., Carobrez, A.P., Lino-de-Oliveira, C., 2008. Frequency of climbing behavior as a predictor of altered motor activity in rat forced swimming test. Neurosci. Lett. 445, 170–173.
Whalen, D.J., Silk, J.S., Semel, M., Forbes, E.E., Ryan, N.D., Axelson, D.A., Birmaher, B., Dahl, R.E., 2008. Caffeine consumption, sleep, and affect in the natural environments of depressed youth and healthy controls. J. Pediatr. Psychol. 33, 358–367.
Yamada, K., Kobayashi, M., Mori, A., Jenner, P., Kanda, T., 2013. Antidepressant-like activity of the adenosine A_{2A} receptor antagonist, istradefylline (KW-6002), in the forced swim test and the tail suspension test in rodents. Pharmacol. Biochem. Behav. 114–115, 23–30.