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Short communication

Caffeine augments the antidepressant-like activity of mianserin and agomelatine in forced swim and tail suspension tests in mice

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ABSTRACT

Background: The main goal of this research was an evaluation of the influence of caffeine on the activity of mianserin and agomelatine.
Methods: The mouse forced swim test and tail suspension test were used to determine the influence of caffeine on the activity of the tested drugs. Drug concentrations in serum and brains were estimated by HPLC.
Results: Caffeine increases the anti-immobility action of mianserin and agomelatine. The observed effects were not associated with changes in the level of drugs in serum or brains.
Conclusion: The synergistic effect of caffeine and the tested drugs may be associated with their summative actions on monoaminergic neurotransmission. Caffeine–mianserin and caffeine–agomelatine interactions might have been of pharmacodynamic origin.

Introduction

Caffeine is one of the most frequently used psychoactive substances [1] which, after oral administration, is rapidly absorbed from the gastrointestinal tract to the bloodstream with bioavailability of 99% [2]. It exerts action on the central nervous system (CNS) after approximately 1 h and the duration of its activity is maintained for 3–4 h. The main mechanism of action of caffeine is its antagonism to adenosine receptors, A_1, A_{2A}, A_{2B} and A_3 [3]. A_1 and A_2 adenosine receptors are mainly located in the hippocampus, cerebral cortex, cerebellar cortex and thalamus. It is known that these areas are responsible for the psychogenic effects of caffeine [3]. According to a recent survey [4], the daily intake of caffeine for the population of the whole world ranges from 70 to 140 mg/day per capita, which corresponds to 1–2 cups of coffee per day. Year on year, caffeine intake is increasing [5].

In connection with an increasing number of depressive-anxiety episodes, and thus the growing number of prescribed centrally acting drugs [6–8], we should consider the problem of taking antidepressant drugs simultaneously with caffeine. There are few scientific reports on the effect of joint administration of caffeine and antidepressants. Therefore, the main goal of this study was to evaluate the influence of caffeine on the activity of mianserin and agomelatine which act through α_2-adrenergic receptors and melatonin receptors, respectively, in the forced swim test (FST) and the tail suspension test (TST). To rule out false positive/negative results, locomotor activity was estimated. Additionally, to evaluate whether the observed effects on animal behavior were due to a pharmacokinetic/pharmacodynamic interaction, the levels of the studied antidepressant drugs and caffeine in mice serum and brain tissues were measured using high-performance liquid chromatography (HPLC).

Materials and methods

Animals

The experiment was carried out on naïve adult male Albino Swiss mice (25–30 g) purchased from a licensed breeder (Kołacz, Warszawa, Poland). The animals were housed in environmentally

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controlled rooms with a 12 h light/dark cycle, in groups of 10 in standard cages under strictly controlled laboratory conditions. They had free access to food and water except for the short time that they were removed from their cages for testing. 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 26/2013) and were performed in accordance with binding European standards related to experimental studies on animal models.

Drug administration

Caffeine (1,3,7-trimethylxanthine, 5 mg/kg, Sigma–Aldrich, Poznań, Poland), and mianserin hydrochloride (10 mg/kg, Sigma–Aldrich) were dissolved in 0.9% NaCl. Agomelatine (20 mg/kg, Sigma–Aldrich) was suspended in a 1% aqueous solution of Tween 80 (POCH, Gliwice, Poland). The solutions of antidepressants were administered intraperitoneally (*ip*) 60 min before behavioral testing, whereas the caffeine solution was administered *ip* 40 min before the tests. The doses and pretreatment schedules were selected based on those reported in the literature and on our previous experiments. All solutions were prepared freshly before the experiment. Animals from the control groups received an *ip* injections of the vehicle (0.9% saline). The volume of the vehicle or drug solutions for *ip* administration was 10 ml/kg.

Forced swim test (FST)

The procedure was carried out on mice, according to the method of Porsolt et al. [9]. Each mouse was placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C. The animals were left in the cylinder for 6 min. The total duration of immobility was recorded during the last 4 min of the 6-min long testing period. The mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above the water level. The immobility time was scored in real time by two observers who were blinded to the treatments.

The results obtained in FST were presented as the arithmetic mean of the immobility time of animals given in seconds ± standard error of the mean (SEM) for each experimental group.

Tail suspension test (TST)

The procedure was carried out on mice, according to the method of Steru et al. [10]. Each mouse was individually suspended by the tail to a vertical bar in a wooden box (30 × 30 cm). The animals were fastened by means adhesive tape fixed 2 cm from the end of the tail 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 to be immobile when it ceased moving its limbs and body, making only those movements necessary to breathe. The immobility time was scored in real time by two observers who were blinded to the treatments.

The results obtained in TST were presented as the arithmetic mean of the immobility time of animals given in seconds ± SEM for each experimental group.

Spontaneous locomotor activity

In order to avoid the risk of obtaining false positive/negative effects in the FST as a result of a possible influence of tested agents on locomotor activity, the spontaneous locomotor activity was measured using an Opto-Varimex-4 Auto-Track animal activity meter (Columbus Instruments, Columbus, OH, USA) which consists of four transparent cages with lids (43 × 43 × 32 cm), a set of four infrared emitters (each emitter has 16 laser beams), and four detectors monitoring animal movements. After *ip* pretreatment with respective drugs or drug combinations (antidepressants and saline were administered *ip* 60 min and caffeine *ip* 40 min before the test), mice were placed individually into cages for 10 min. Spontaneous locomotor activity was evaluated between the 2nd and the 6th min, which corresponded with the time interval analyzed in the FST.

The results obtained in this test were presented as the arithmetic average distance that a mouse traveled (in cm) ± SEM for each experimental group.

Determination of antidepressants in serum and brain tissue

Sixty minutes following administration of antidepressant drugs with or without 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 10000 rpm for 10 min and serum was collected into polyethylene tubes and frozen at −25 °C. Immediately after decapitation, the brains were dissected from the skull, washed with 0.9% NaCl and also frozen at −25 °C.

The serum and brain concentrations of the studied antidepressants were assayed by the high performance liquid chromatography (HPLC) method. The brains were homogenized in distilled water (1:4, w/v) with a TH220 tissue homogenizer (Omni International, Inc., Warrenton, VA, USA). For agomelatine, the extraction from 1 ml of brain homogenate was performed using a 5 ml of mixture of dichloromethane:hexane:isoamyl alcohol (39.5:59.5:1 v/v/v). Carbamazepine (100 ng/ml) was used as an internal standard (IS). Agomelatine was extracted from 200 μl of serum after the addition of the appropriate IS and 1 ml of dichloromethane as the extraction reagent. In turn, IS was added to 1 ml of brain homogenate containing this drug. After the addition of 1 ml of the concentrated NaCl solution (10 g/50 ml), the samples were vortexed for 15 s and 5 ml of the extraction reagent was added. Next, the samples were shaken for 20 min and centrifuged for 15 min at 3000 rpm. After the centrifugation, the organic layer was transferred into a conical glass tube and evaporated to dryness at 37 °C under a gentle stream of nitrogen in a water bath. The residue was dissolved with 100 μl of methanol and 50 μl of this solution was injected into the HPLC system.

For mianserin, the extraction from serum and brain homogenates was performed using a mixture of ethyl acetate:hexane (30:70, v/v). Amitriptyline (2 μg/ml) was used as IS. In the case of mianserin, IS was added to samples of serum (200 μl) and brain homogenate (0.5 ml) containing this drug and the samples were alkalized with 100 and 250 μl of 4 M NaOH, respectively. Next, 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 layer was transferred to a new tube containing a 200 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). A 50 μl aliquot of this solution was injected into the HPLC system.

The HPLC system (Thermo Separation Products, San Jose, CA, USA) consisted of a P100 isocratic pump, a UV100 variable-wavelength UV/VIS detector, a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 50 μl sample loop, and a Chromjet SP4400 computing integrator. All analyses were performed on a 250 × 4 mm LiChrospher®100 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 65:35 (v/v) for agomelatine, and 60:40 (v/v) for mianserin, 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 mianserin. The calibration curves constructed by plotting the ratio of the peak heights of the studied drug to IS *vs.* the 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 (a coefficient of variation less than 10%). The extraction efficiencies of the analyzed compounds and internal standards ranged from 66 to 97%. Antidepressant concentrations were expressed in ng/ml of serum or ng/g of wet brain tissue.

Determination of caffeine in serum and brain tissue

Forty min following the administration of caffeine with or without mianserin or agomelatine, mice were decapitated to collect biological material for pharmacokinetic studies. The blood and brains for the determination of the caffeine concentration were prepared as indicated above.

Serum and brain concentrations of caffeine were assayed by the liquid chromatography–mass spectrometry (LC–MS) method. The brains were homogenized in 0.1 M perchloric acid (1:4, w/v) with a PRO 200 Bio-Gen Series tissue homogenizer (Pro Scientific Inc., Oxford, UK). After shaking for 0.5 h and centrifuging for 15 min (3000 rpm), samples were filtered through nylon syringe filters (nominal pore diameter 0.45 μm), transferred to 2 ml vials and subjected to LC–MS analysis. The undertaken sample preparation methods constituted modifications of the procedures performed by Carey and DePalma [11].

To plasma samples, methanol (1:4, v/v) was added in order to extract the caffeine. All samples were shaken for 0.5 h and then filtered through a nylon syringe filter (nominal pore diameter 0.45 μm), evaporated to dryness, resuspended in 0.5 ml of methanol and subjected to LC–MS analysis.

The LC–MS system (Agilent Technologies, Santa Clara, CA, USA) consisted of a binary pump, a thermostate column, an autosampler and a PDA detector (Series 1200) coupled with a mass spectrometer with an ESI dual-spray source (G3250AALC/6210 MSD TOF). All analyses were performed on a 150 × 2.1 mm Zorbax RP-18 Stable Bond column.

The separation process was performed on a chromatographic column in the presence of a gradient solvent mixture, composed of solvent A (water + 0.1% formic acid) and solvent B (acetonitrile + 0.1% formic acid). For brain samples, the gradient was set as follows: 0–10 min from 2 to 10% of B in A, 10–27 min from 10 to 40% of B in A, 27–29 min from 40 to 95% holding the gradient from 29 to 31 min, and then returning to the initial settings: 31–31.5 min from 95 to 2% of B in A, and maintaining the analysis period until 40th min.

For plasma, due to the less complex composition of the samples, the following conditions were applied: 0–10 min from 2 to 10% of B in A, 10–21 min from 10 to 40% of B in A, 21–23 min holding the gradient at 40% of B in A, then decreasing the polarity at 23–27 min, from 40 to 95% and introducing the initial settings: 27–28 min 95–2% of B in A. The run time was set as 40 min, the flow rate as 0.25 ml/min and the temperature of the thermostat as 25 °C. The UV detector registered the spectra in the following wavelengths: 260 nm and 280 nm.

A calibration curve for caffeine was prepared based on several dilutions of a stock solution prepared by dissolving 5.08 mg of the standard in 50 ml of methanol (0.08, 0.04, 0.016, 0.004, 0.0016, 0.008 mg/ml, with an injection volume of 10 μl). The inter-day and intra-day values (measured by a triple injection) were determined for the method. Caffeine concentrations were expressed in ng/ml of serum or ng/g of wet brain tissue.

Statistical analysis

Statistical analysis of the results obtained in the FST, TST and locomotor activity was carried out using two-way ANOVA with Bonferroni's *post hoc* test. The first main factor assessed in two-way ANOVA was the effect of caffeine and the second was the effect of a tested drug. The concentrations of the tested antidepressant drugs in blood and brains of mice in the presence and absence of caffeine were compared using the Student's *t*-test, p values less than or equal to 0.05 were considered statistically significant.

Results

FST

The influence of caffeine on the antidepressant activity of tested drugs in FST

Effect of combined administration of caffeine and mianserin in FST. The effect of the combined administration of caffeine and mianserin on the total duration of the immobility time in mice is shown in Fig. 1A. Caffeine (5 mg/kg) injected in combination with mianserin (10 mg/kg) significantly reduced the immobility time in the FST in mice (Fig. 1A). Mianserin (10 mg/kg) and caffeine (5 mg/kg) given alone had no effect on the immobility time (Fig. 1A).

Two-way ANOVA demonstrated a significant effect of mianserin [$F(1,36) = 20.51$, $p < 0.0001$], a significant effect of caffeine [$F(1,36) = 25.98$, $p < 0.0001$], and significant interaction between mianserin and caffeine [$F(1,36) = 9.29$, $p = 0.0043$].

Effect of combined administration of caffeine and agomelatine in FST. The effect of the combined administration of caffeine and agomelatine on the total duration of the immobility time in mice is shown in Fig. 1B. Caffeine (5 mg/kg) injected in combination with agomelatine (20 mg/kg) significantly reduced the immobility time in the FST in mice (Fig. 1B). Agomelatine (20 mg/kg) and caffeine (5 mg/kg) given alone had no effect on the immobility time (Fig. 1B).

Two-way ANOVA demonstrated a significant effect of agomelatine [$F(1,36) = 12.33$, $p = 0.0012$], a significant effect of caffeine [$F(1,36) = 10.23$, $p = 0.0029$], and significant interaction between agomelatine and caffeine [$F(1,36) = 7.93$, $p = 0.0078$].

TST

The influence of caffeine on the antidepressant activity of tested drugs in TST

Effect of combined administration of caffeine and mianserin in TST. The effect of the combined administration of caffeine and mianserin on the total duration of the immobility time in mice is shown in Fig. 1C. Caffeine (5 mg/kg) injected in combination with mianserin (10 mg/kg) significantly reduced the immobility time in the TST in mice (Fig. 1C). Mianserin (10 mg/kg) and caffeine (5 mg/kg) given alone had no effect on the immobility time (Fig. 1C).

Two-way ANOVA demonstrated a significant effect of mianserin [$F(1,36) = 11.84$, $p = 0.0015$], a significant effect of caffeine [$F(1,36) = 50.72$, $p < 0.0001$], and significant interaction between mianserin and caffeine [$F(1,36) = 25.27$, $p < 0.0001$].

Effect of combined administration of caffeine and agomelatine in TST. The effect of the combined administration of caffeine and agomelatine on the total duration of the immobility time in mice is shown in Fig. 1D. Caffeine (5 mg/kg) injected in combination with agomelatine (20 mg/kg) significantly reduced the immobility time in the TST in mice (Fig. 1D). Agomelatine (20 mg/kg) and caffeine

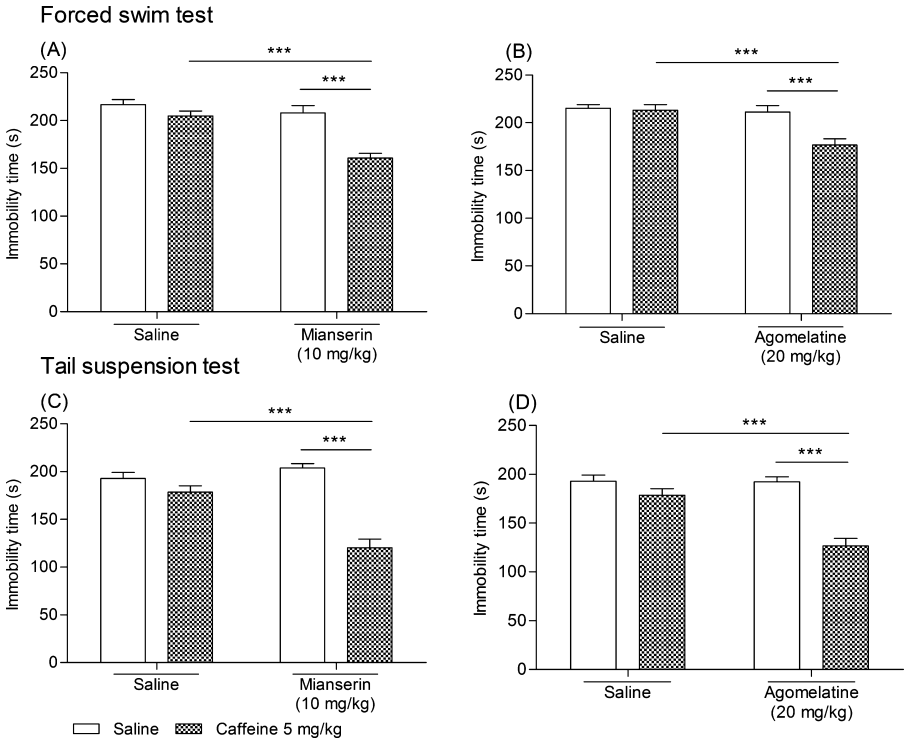

Fig. 1. Effect of combined administration of caffeine and antidepressants in the FST (A,B) and TST (C,D) in mice. Before the tests, caffeine was administered *ip* 40 min, and mianserin, agomelatine and saline were administered *ip* 60 min. The values represent mean ± SEM (n = 10 per group). ***$p < 0.001$ (two-way ANOVA followed by Bonferroni's *post hoc* test).

(5 mg/kg) given alone had no effect on the immobility time (Fig. 1D).

Two-way ANOVA demonstrated a significant effect of agomelatine [$F(1,36) = 16.34$, $p = 0.0003$], a significant effect of caffeine [$F(1,36) = 37.79$, $p < 0.0001$], and significant interaction between agomelatine and caffeine [$F(1,36) = 15.48$, $p = 0.0004$].

Spontaneous locomotor activity

Effect of combined administration of caffeine and antidepressants on locomotor activity in mice

The effect of the combined administration of caffeine and the tested antidepressant drugs on spontaneous locomotor activity in mice is shown in Table 1.

Neither caffeine nor antidepressants (mianserin, agomelatine) administered either alone or combined together resulted in a statistically significant increase in locomotor activity in mice (Table 1). A statistically significant shortening of the distance covered by the mouse was observed in the group treated with mianserin alone and mianserin with caffeine (Table 1).

Two-way ANOVA demonstrated:

(A) A significant effect of mianserin [$F(1,26) = 44.92$, $p < 0.0001$], no effect of caffeine [$F(1,26) = 3.54$, $p = 0.0713$], and no interaction [$F(1,26) = 0.06$, $p = 0.8039$].
(B) No effect of agomelatine [$F(1,25) = 1.71$, $p = 0.2035$], a significant effect of caffeine [$F(1,25) = 10.43$, $p = 0.0035$], and no interaction [$F(1,25) = 0.00$, $p = 0.9764$].

Table 1
Effect of treatments on spontaneous locomotor activity in mice.

	Treatment (mg/kg)	Distance traveled between 2nd and 6th min (cm)
(A)	Saline + saline	713.9 ± 62.96
	Caffeine 5 + saline	863.5 ± 94.38
	Mianserin 10 + saline	260.9 ± 37.21***
	Caffeine 5 + mianserin 10	375.3 ± 71.40***
(B)	Saline + saline	538.0 ± 60.10
	Caffeine 5 + saline	760.4 ± 88.98
	Agomelatine 20 + saline	445.1 ± 49.83
	Caffeine 5 + agomelatine 20	671.7 ± 77.15

Antidepressants and saline were administered *ip* 60 min and caffeine *ip* 40 min before the test. Distance traveled was recorded between the 2nd and the 6th min of the test. Each experimental group consisted of 7–8 animals. Data are presented as the means ± SEM.
*** $p < 0.001$ *vs.* control group (two-way ANOVA followed by Bonferroni's *post hoc* test).

Pharmacokinetic studies

The effect of caffeine on the serum and brain concentrations of the tested antidepressants in mice is shown in Table 2. In the case of the co-administration of caffeine and mianserin or agomelatine, no changes in drug concentrations were observed in serum and the brain.

The effect of the tested drugs on serum and brain concentrations of caffeine in mice is shown in Table 3. In the case of joint administration of caffeine and mianserin, a significant decrease in caffeine concentrations was noted both in serum ($p = 0.0035$) and the brain ($p < 0.0003$). No statistically significant changes were obtained in terms of the concentration of caffeine in the group treated with caffeine and agomelatine *vs.* the control group.

Discussion

Mianserin and agomelatine belong to a group of antidepressants which substantially differ from other antidepressant drugs with respect to their action mechanism. Both of these substances

Table 2
Effect of caffeine on the concentrations of antidepressants in mouse serum and brain.

Treatment (mg/kg)	Drug concentration			
	Serum (ng/ml)		Brain (ng/g)	
Mianserin 10 + saline	137.2 ± 13.10		1380.0 ± 130.00	
Mianserin 10 + caffeine 5	189.3 ± 24.57	$p = 0.0775$	1830.0 ± 200.00	$p = 0.0803$
Agomelatine 20 + saline	34.45 ± 21.13		29.96 ± 17.29	
Agomelatine 20 + caffeine 5	12.75 ± 3.74	$p = 0.3253$	12.45 ± 1.49	$p = 0.3263$

Antidepressants were administered *ip* 60 min, and caffeine 40 min before decapitation. Each experimental group consisted of 10 animals. Results are presented as mean values ± SEM. The data were analyzed by the Student's *t*-test.

produce antidepressant activity by interfering with specific receptors. Mianserin acts by blocking α_2-andrenergic autoreceptors on serotoninergic neurons and consequently potentiates 5-HT neurotransmission [12]. Furthermore, mianserin has affinity for α_1-adrenergic receptors and blocks postsynaptic 5-HT_2 receptors which directs the action of serotonin on postsynaptic receptors 5-HT_{1A}, whose activation is essential for antidepressant activity. It has also been demonstrated that mianserin increases noradrenaline and dopamine release in the CNS [12]. The second drug, agomelatine, is a relatively new antidepressant, which has agonistic activity toward MT_1 and MT_2 melatonin receptors. Agomelatine also blocks serotonin receptors type 5-HT_{2a} and 5-HT_{2c}, increasing indirectly the release of dopamine and noradrenaline in the prefrontal cortex [13,14].

The results of the our study showed that a non-effective dose of caffeine increases the antidepressant-like activity of mianserin and agomelatine also at non-effective doses, which shortened the immobility period of mice both in the FST and TST. The synergistic effect of caffeine on the activity of mianserin may be associated with the summative actions of both these substances on monoaminergic transmission in the CNS. Mianserin, which inhibits α_2-adrenergic receptors and exerts pronounced antagonistic properties at serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors, increases serotonergic, noradrenergic and dopaminergic neurotransmission in the CNS [12]. Caffeine is characterized by a stimulating influence on the cerebral cortex which is related to the release of various neurotransmitters in the CNS, such as acetylcholine, γ-aminobutyric acid (GABA), glutamate, dopamine, noradrenaline and 5-HT, which is linked to the non-selective antagonist effect of caffeine on adenosine A_1, A_2 and A_3 receptors [15]. It should be noted that results obtained in the present study demonstrated a decreased locomotor activity in mice from both groups of subjects receiving only mianserin, as well as the group treated with caffeine and mianserin. This effect is presumably due to the inhibitory effect of mianserin on the H_1 histamine receptors [12]. Moreover, statistical analysis of the results of the locomotor activity test indicated that caffeine has a tendency to potentiate mice activity, as expected from a psychostimulant drug. However, in the case of the application of caffeine in combination with mianserin, no increase in spontaneous activity in animals was noted and the interaction between these two substances was found to be statistically insignificant.

The present study was designed to investigate the ability of caffeine to modify the anti-immobility action of agomelatine in the FST and TST in mice. The results of our research indicate that caffeine increased the effect of agomelatine (both at a non-effective dose), which is manifested in a shortened duration time for the immobility period of animals. Statistical analysis of results obtained in locomotor activity tests showed a tendency to increased locomotor activity of animals as a result of caffeine co-administration, but this trend did not reflect in the interaction between caffeine and agomelatine, which is insignificant. Synergism observed in the FST and TST is probably caused by caffeine, *inter alia*, through noradrenergic and dopaminergic transduction [15] which, in addition to the effect on melatonin receptors, is also responsible for the antidepressant effect of agomelatine [13,14].

Moreover, because caffeine is an antagonist of adenosine [3], it is believed that caffeine consumption is responsible for the decrease in melatonin levels [16]. It has been shown that in humans caffeine has practically an immediate effect on melatonin concentration. In patients treated with caffeine at a daily dose of 50–200 mg, melatonin concentration is lower than in patients receiving a placebo [16]. Probably, the inhibitory effect of caffeine at A_{2B} receptors located in the pineal gland is responsible for these changes [17]. The fact that adenosine increases melatonin synthesis in the pineal gland in a dose-dependent manner supports this hypothesis [17]. Furthermore, studies carried out by Babey et al. [18] have shown that caffeine blocks adenosine-stimulated melatonin production.

To verify the data achieved in the behavioral tests and to evaluate the potential pharmacokinetic/pharmacodynamic interactions between caffeine and the tested agents, the concentrations of caffeine, mianserin and agomelatine in murine serum and brain were evaluated. The probability of pharmacokinetic interaction between caffeine and antidepressants is very high, due to the fact that caffeine is metabolized by the CYP1A2 isoenzyme of cytochrome P450 [19], which is also involved in the biotransformation of most drugs used in the treatment of depressive disorders, *e.g.* imipramine, desipramine, citalopram, fluoxetine, paroxetine, and mianserin [20].

Pharmacokinetic studies demonstrated that the effects observed in behavioral tests were not associated with changes in the level of mianserin and agomelatine in the blood and brain of animals. Concomitant administration of caffeine did not result in a

Table 3
Effect of antidepressants on the concentrations of caffeine in mouse serum and brain.

Treatment (mg/kg)	Caffeine concentration			
	Serum (ng/ml)		Brain (ng/g)	
Mianserin 10 + saline	671.5 ± 67.14		1785.0 ± 148.80	
Mianserin 10 + caffeine 5	419.5 ± 33.33	$p = 0.0035$	976.5 ± 102.20	$p = 0.0003$
Agomelatine 20 + saline	671.5 ± 67.14		1785.0 ± 48.80	
Agomelatine 20 + caffeine 5	744.7 ± 82.90	$p = 0.5016$	1509.0 ± 32.40	$p = 0.1830$

Antidepressants were administered *ip* 60 min, and caffeine 40 min before decapitation. Each experimental group consisted of 10 animals. Results are presented as mean values ± SEM. The data were analyzed by the Student's *t*-test.

significant increase/decrease in the concentration of both studied antidepressants in serum and brain homogenates; rather, in the case of the joint administration with mianserin, a significant decrease in the level of caffeine was observed. In this combination, the results suggest that the caffeine–mianserin interaction might have been partially pharmacodynamic and pharmacokinetic in nature, but the caffeine–agomelatine interaction could have occurred in the pharmacodynamic phase.

In conclusion, FST outcomes in our preliminary studies indicated a synergistic action of caffeine in combination with mianserin and agomelatine which was not connected with hyperlocomotion. HPLC analysis of mianserin and agomelatine concentrations and LC-MS analysis of caffeine level in murine serum and brain homogenates indicated that the observed synergism of action of caffeine and agomelatine was related to a pharmacodynamic rather than pharmacokinetic interaction, because there were no changes in either agomelatine or caffeine concentrations in either of the examined tissues. In the case of concomitant administration of caffeine and mianserin, concentration analyses showed the reduction in levels of caffeine in both serum and brain. This may be due to the severity of the hepatic metabolism of caffeine in the presence of mianserin, which indicates an interaction in the pharmacokinetic phase. Synergism of antidepressant-like action between caffeine and mianserin obtained in animal behavioral tests showed that these interactions also occurred in the pharmacodynamic phase.

Conflict of interest

The authors declare that they have no conflict of interest.

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