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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