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The influence of caffeine on the activity of moclobemide, venlafaxine, bupropion and milnacipran in the forced swim test in mice

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ABSTRACT

Aims: Worrying data indicate that excessive caffeine intake applies to patients suffering from mental disorders, including depression. It is thus possible to demonstrate the usefulness of caffeine and its derivatives in the treatment of depression. The main goal of the present study was to evaluate the influence of caffeine (5 mg/kg) on the activity of moclobemide (1.5 mg/kg), venlafaxine (1 mg/kg), bupropion (10 mg/kg), and milnacipran (1.25 mg/kg). Moreover, we assessed the influence of caffeine on their serum and brain levels using high-performance liquid chromatography.
Main methods: The experiment was carried out on naïve adult male Albino Swiss mice. Caffeine and tested drugs were administered intraperitoneally. The influence of caffeine on the activity of selected antidepressant drugs was evaluated in forced swim test (FST). Locomotor activity was estimated to verify and exclude false positive/negative results. To assess the influence of caffeine on the levels of studied antidepressant drugs, their concentrations were determined in murine serum and brains using high-performance liquid chromatography.
Key findings: Caffeine potentiated activity of all antidepressants examined in FST and the observed effects were not due to the increase in locomotor activity in the animals. Only in the case of co-administration of caffeine and milnacipran an increased milnacipran concentration in serum was observed without affecting its concentration in the brain.
Significance: Caffeine potentiates the activity of antidepressant drugs from different chemical groups. The interactions of caffeine with venlafaxine, bupropion and moclobemide occur in pharmacodynamic phase, whereas the interaction of caffeine–milnacipran occurs, at least partially, in pharmacokinetic phase.

1. Introduction

Caffeine is the most widely used behavioral active drug in the world [58] which acts as an antagonist of the adenosine receptors A_1, A_2 and A_3 [21,23,54]. Higher doses of caffeine inhibit phosphodiesterase, block the receptors for γ-aminobutyric acid type A ($GABA_A$), and cause the release of intracellular Ca^{2+} [40]. Caffeine is characterized by a stimulating influence on the cerebral cortex which is related to the release of various neurotransmitters in the central nervous system [14,22,26,29,45,59,69]. Effects of caffeine activity are a common issue to investigate in the scientific world. A lot of research that aims at defining the effect of caffeine on human body is carried out. The predominant outcomes suggest that caffeine has an impact on mood boost [35,40], as well as on consciousness enhancement, acquiring and processing information, and reaction time and attention [6].

Caffeine is present in many drinks and its content in a cup of coffee is as high as 100 mg. Average daily consumption of caffeine amounts to 3 mg/kg a day. Caffeine is very frequently an ingredient of analgesics, appetite inhibition drugs or additive of stimulating preparations [42,43]. Caffeine consumption rises every year. Worrying data indicate that excessive caffeine intake applies to patients suffering from mental disorders. Many patients with depression feel continuous exhaustion and hence consume significant quantities of caffeine [1], which results in an advantageous effect [66]. It is estimated that this issue affects about 22% of hospitalized patients who were diagnosed with mental disorders compared with 9% for healthy people [30]. Due to an increasing number of cases diagnosed with mental illnesses, including depression, and a widespread use of antidepressants with various action mechanisms, it seems to be important to test the interactions between caffeine and the antidepressant drugs. In our recent study we showed that caffeine

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increased the effect of typical antidepressant drugs, such as imipramine and its metabolite — desipramine (tricyclic antidepressant — TCA), fluoxetine, escitalopram and paroxetine (selective serotonin reuptake inhibitors, SSRI) and reboxetine (selective noradrenaline reuptake inhibitor, NRI) (unpublished data). Therefore, the main goal of this study was to evaluate the influence of caffeine on the activity of antidepressants acting through other mechanisms, such as moclobemide, venlafaxine, bupropion and milnacipran in the forced swim test (FST). To verify and exclude false-positive or false-negative results locomotor activity was estimated. Additionally, to evaluate whether the observed animals' behavior effects were consequent to a pharmacokinetic/pharmacodynamic interaction, concentrations of the studied antidepressant drugs in mice serum and brain tissue homogenates were measured using high-performance liquid chromatography (HPLC).

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 (Kolacz, 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, relative humidity about 45–55%. Throughout the study, the animals were given ad libitum access to water and food. The experiment began after at least 1-week acclimation period in the laboratory conditions and was conducted between 8 a.m. and 3 p.m. to minimize circadian influences. Each experimental group consisted of 8–12 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 26/2011, 28/2011 and 26/2013) 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 (1,3,7-trimethylxanthine; 5 mg/kg, Sigma-Aldrich), moclobemide (1.5 mg/kg, Sigma-Aldrich, Poznań, Poland), milnacipran hydrochloride (1.25 mg/kg, Abcam, Cambridge, UK), venlafaxine hydrochloride (1 mg/kg, Sigma-Aldrich, Poznań, Poland) and bupropion hydrochloride (10 mg/kg, Abcam, Cambridge, UK) were dissolved in 0.9% NaCl. The solutions of antidepressants were administered intraperitoneally (*ip*) 60 min before behavioral testing whereas caffeine solution was administered *ip* 40 min before the tests. The doses and pretreatment schedules were selected on those reported in the literature and on the basis of the results of our previous experiments [49,52,57,68]. All solutions were prepared immediately prior to the experiment. Animals from the control groups received *ip* injections of the vehicle (0.9% saline). The volume of the vehicle or drug solutions for *ip* administrations was 10 ml/kg.

2.3. Forced swim test (FST)

The procedure was carried out on mice, according to the method of Porsolt et al. [53]. Each mouse was placed individually into the glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water at 23–25 °C, which was exchanged for clean after each test (each mice). The animals were left in the cylinder for 6 min. The total duration of immobility was real-time recorded by cumulative stopwatches 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 the movements necessary to keep its head above the water level.

The results obtained in the FST were 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.

2.4. Spontaneous locomotor activity

In order to avoid the risk of obtaining the false positive/negative effects in the FST caused by a possible influence of tested agents on the locomotor activity, the spontaneous locomotor activity was measured using an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, USA). This actimeter 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. After *ip* pretreatment with respective drugs or drug combinations and after a given time period mice were placed individually into the cages for 10 min. Spontaneous locomotor activity was evaluated between the 2nd and the 6th minute, which corresponds 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) ± S.E.M. for each experimental group.

2.5. Determination of antidepressants in the serum and brain homogenates

Sixty minutes following administration of tested antidepressant drugs with or without caffeine, unanesthetized 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 0.9% NaCl and also frozen at −25 °C.

Serum and brain concentrations of the studied antidepressants were assayed by HPLC. The brains were homogenized in distilled water (1:4, w/v) with a tissue homogenizer TH220 (Omni International, Inc., Warrenton, VA, USA). For all studied drugs, the extraction from serum and brain homogenates was performed using the mixture of ethyl acetate:hexane (30:70, v/v). Internal standard (IS) for venlafaxine was reboxetine (500 ng/ml), for milnacipran — bupropion (1 μg/ml), for bupropion — milnacipran (1 μg/ml) and for moclobemide — venlafaxine (1 μg/ml).

To isolate venlafaxine, milnacipran, bupropion, and moclobemide from serum (200 μl) or brain homogenate (1 ml) containing these drugs an appropriate IS was added and the samples were alkalized with 100 μl of 4 M NaOH. Then the samples were extracted twice with 3 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 a new tube containing a 100 μl solution of 0.1 M H_2SO_4 and methanol (90:10 v/v) or 0.1 M HCl (for milnacipran and bupropion), and the mixture was shaken for 0.5 h and then centrifuged for 15 min (3000 rpm). The organic layer was discarded and a 50 μl aliquot of the acidic 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.

The analyses of bupropion and milnacipran were performed on a 250 × 4.6 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 separation of moclobemide and venlafaxine was conducted using 250 × 4.6 mm Supelcosil LC-CN column with a particle size of 5 μm (Sigma Aldrich, Germany) protected with a guard column (4 × 4 mm) with the same packing material. The mobile phase consisted of 50 mM potassium dihydrogen phosphate and acetonitrile mixed at a ratio of 85:15 (v/v)