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Original article

The effect of chronic co-treatment with risperidone and novel antidepressant drugs on the dopamine and serotonin levels in the rats frontal cortex

Katarzyna Kamińska*, Anna Górska, Karolina Noworyta-Sokołowska, Adam Wojtas, Zofia Rogóż, Krystyna Gołembiowska

Institute of Pharmacology, Polish Academy of Sciences, Department of Pharmacology, Kraków, Poland

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ABSTRACT

Background: Preclinical and clinical studies have suggested a beneficial effect of combination treatment with atypical antipsychotic drugs and antidepressants (ADs) in schizophrenia and in drug-resistant depression.
Methods: In the present study, we investigated the effect of chronic administration of risperidone and ADs (escitalopram or mirtazapine), given separately or jointly on the extracellular levels of dopamine (DA) and serotonin (5-HT) in the rat frontal cortex. The animals were administered risperidone (0.2 mg/kg) and escitalopram (5 mg/kg) or mirtazapine (10 mg/kg) repeatedly for 14 days. The release of monoamines in the rat frontal cortex was evaluated using a microdialysis, and DA and 5-HT levels were assayed by HPLC. We also measured the locomotor activity, catalepsy and recognition memory in these rats.
Results: Chronic risperidone treatment (0.2 mg/kg) increased the extracellular levels of DA and 5-HT. Co-treatment with risperidone and escitalopram (5 mg/kg) or mirtazapine (10 mg/kg) more efficiently increased the release of 5-HT but not DA in the rat frontal cortex, as compared to drugs given alone. Moreover, risperidone, escitalopram and mirtazapine given alone or in combination significantly decreased the locomotor activity and only mirtazapine increased the catalepsy evoked by risperidone. Combined treatment with risperidone and ADs impaired recognition memory in these rats.
Conclusions: The obtained results suggest that chronic co-administration of risperidone and escitalopram or mirtazapine more efficiently increased 5-HT release in the rat frontal cortex as compared to drugs given alone and suggest that this effect may be of importance to the pharmacotherapy of schizophrenia and drug-resistant depression.

Introduction

Antidepressants (ADs) are often combined with antipsychotic drugs to treat depression. Preclinical and clinical data suggest that joint administration of the atypical antipsychotic risperidone (RIS) and ADs with different pharmacological mechanisms of action may increase their antidepressant efficacy [1,2]. In addition, novel ADs given as adjunct to antipsychotic drugs are more effective in decreasing negative symptoms of schizophrenia. This drug combination may also overcome side effects associated with high doses of RIS [3].

RIS, a second generation antipsychotic drug at low doses blocks serotonin 5-HT_{2A} while at higher ones inhibits dopamine D_2 receptors [4]. This drug is ca. 20–50 times more potent in its binding to 5-HT_{2A} serotonin receptors than to α_1 and α_2-adrenergic, dopamine D_2, histamine H_1 receptors [4,5]. Beside the antipsychotic mechanism, the blockade of 5-HT_{2A} receptors in cortical regions and inhibition of glutamatergic neurotransmission [6] implies also an anxiolitytic and antidepressant effect of this drug [7]. On the other hand, escitalopram (ESC), a selective serotonin reuptake inhibitor, enhances serotonergic neurotransmission. ESC does not block dopaminergic and noradrenergic receptors thus, it does not cause attention deficits [8,9]. It has also been shown that mirtazapine (MIR), a novel AD, by blocking α_2-adrenoreceptors enhances noradrenergic and serotonergic neurotransmission [10]. Moreover, it does not inhibit noradrenaline and 5-HT transporters. MIR also binds to 5-HT_2 and 5-HT_3 receptors and displays very low affinity for dopamine (DA) receptors and high affinity for histamine H_1 ones [11].

In our previous study, we investigated the acute effect of MIR, fluoxetine (FLU) and ESC given separately or jointly with

* Corresponding author.
E-mail address: katkam@if-pan.krakow.pl (K. Kamińska).

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risperidone on extracellular levels of dopamine (DA), serotonin (5-HT) and noradrenaline (NA) in the rat frontal cortex and nucleus accumbens [12–14]. It was found that FLU (10 mg/kg) and MIR (10 and 20 mg/kg) potentiated risperidone (1 mg/kg) effect on DA, 5-HT and NA release in the rat frontal cortex [12–14]. ESC (5 and 10 mg/kg) enhanced risperidone (0.2 and 1 mg/kg) action on DA and 5-HT release in the rat frontal cortex and nucleus accumbens [12–14]. We also investigated the antidepressant and anxiolytic effect of acute administration of MIR, FLU, ESC and risperidone with the use of the forced swimming test (FST) and elevated plus maze test (EPMT), respectively [15]. It was found that risperidone (0.05 and 0.1 mg/kg) increased the antidepressant-like effect of ESC (1 mg/kg) in the FST in rats [14]. Joint administration of risperidone (0.1 mg/kg) and ESC (5 mg/kg) or MIR (1 and 2.5 mg/kg) was more efficient in producing the anxiolytic effect in EPMT than a single treatment with any of these drugs [15].

The present study was undertaken to determine the efficacy of chronic treatment with ESC or MIR and risperidone given separately or jointly on the DA and 5-HT release in the rat frontal cortex in more clinically relevant conditions. We chose to administer 0.2 mg/kg of risperidone due to potent sedation that followed administration of 1 mg/kg. Selected doses of escitalopram (5 mg/kg) and mirtazapine (5 mg/kg) were chosen due to highly repeatable effect on extracellular monoamine levels.

Materials and methods

Animals

All experiments were performed on male Wistar-Han rats (280–350 g) derived from Charles River (Germany). Animals were kept in temperature (23 °C) and humidity (55 ± 10%) – controlled rooms with a 12-h light-dark cycle (the light on at 7 a.m.), and free access to water and food. The experimental procedures were conducted in a strict accordance with Polish legal regulations concerning experiments on animals (Dz. U. 05.33.289). The experimental protocols were approved by Local Ethics Commission for Experimentation on Animals. The animals were not kept in enriched environment.

Drug administration

Animals were administered intraperitoneally (*ip*) risperidone (RIS, Tocris Bioscence, Bristol, UK) at a dose of 0.2 mg/kg, escitalopram oxalate (ESC, Tocris, Bristol, UK) at doses of 5 mg/kg and mirtazapine (MIR, Tocris Bioscience, Bristol, UK) at a dose of 10 mg/kg, a combination of RIS (0.2 mg/kg) with ESC (5 mg/kg) or a combination of RIS (0.2 mg/kg) with MIR (5 mg/kg) once a day for 14 days. ESC was dissolved in a 0.9% NaCl while MIR was suspended in a 1% aqueous solution of Tween 80. RIS was dissolved in 0.1 M tartaric acid solution adjusted to pH 6–7 with 0.1 M NaOH. All the chemicals used for high performance liquid chromatography (HPLC) came from Merck (Warszawa, Poland).

Microdialysis

Twenty four hours after the last injection rats were anesthetized with ketamine (75 mg/kg *im*) and xylazine (10 mg/kg *im*), placed in a stereotactic apparatus (David Kopf Instruments, Tujunga, USA) and subsequently microdialysis probes (3 mm, Agntho's, Sweden) were implanted in the rat frontal cortex with coordinates (mm) A + 2.8, L + 0.8, V – 6.0 from the dura. Twenty four hours after implantation, probe inlets were connected to a syringe pump (CMA, Sweden) which delivered an artificial cerebrospinal fluid (aCSF) composed of (mM): NaCl 147, KCl 4.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0 at a flow rate of 2 μl/min. Four baseline samples were collected every 20 min after the washout period (2 h). Appropriate drugs were then administered and dialysate fractions were collected for 180 min. At the end of the experiment, the rats were sacrificed and their brains were histologically examined to validate probe placement.

Analytical procedure

Dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) were analyzed by HPLC with coulochemical detection. Chromatography was performed using an Ultimate 3000 System (Dionex, USA), coulochemical detector Coulochem III (model 5300, ESA, USA) with a 5020 guard cell, a 5014 B microdialysis cell and a Hypersil Gold-C18 (Thermo Scientific, USA) analytical column (3 × 100 mm). The mobile phase was composed of 0.1 M potassium phosphate buffer adjusted to pH = 3.8, 0.5 mM EDTA, 96 mg/L 1-octanesulfonic acid sodium salt, and a 2% methanol. The flow rate during analysis was 0.7 ml/min. The applied potential of a guard cell was +600 mV, while those of microdialysis cell was E1 = −50 mV, E2 = +300 mV and a sensitivity was set at 50 nA/V. The chromatographic data were processed by Chromeleon v. 6.80 (Dionex, USA) software run on a personal computer. The limit of detection of DA and 5-HT in dialysates was 0.02 pg/10 μl for DA and 0.006 pg/10 μl for 5-HT.

Locomotor activity test

The locomotor activity was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, Columbus, USA) connected to a compatible IBM-PC. Each chamber (43 cm × 43 cm × 21 cm), equipped with a 220-lx house light, was made of transparent acrylic plastic (all 6 sides) and was housed in a light – and soundproof wooden cubicle. The corner brackets were made of a stainless steel. Each cage was surrounded by a 15 × 15 array of photocell beams located 3 cm from the floor surface. Interruptions of the photobeams indicated horizontal locomotor activity, defined as a distance traveled and expressed in cm. The vertical locomotor activity defined as an ambulation time was expressed in s. Measurements of locomotor activity began 24 h after last drug administration.

Catalepsy test

Catalepsy induced by chronic administration of RIS (0.2 mg/kg) alone or its co-treatment with MIR (10 mg/kg) or ESC (5 mg/kg) was determined according to a six-test method described by Simon et al. [16]. The degree of catalepsy was evaluated at 24 h after the last drugs injection. A positive response in each trial (when the animals maintained an abnormal position for at least 10 s) was regarded as 1 point (lower bar), or 2 points (higher bar) for forepaw, and thus each rat could score a maximum of 6 points.

Novel object recognition test

On the day of the experiment rats were transferred to the laboratory, labeled and weighed, and, thereafter, left to acclimate to the new environment for approximately 2 h before the test started. In the first, i.e. introductory session, each rat was placed in a white plastic box (40 cm × 60 cm × 50 cm). Objects to discriminate were: a black metal box (5 cm × 14 cm) and a green glass cone (5 cm × 14 cm). Objects were placed in two opposite corners with the center of the object 24 cm from the corner. A rat was placed in the middle of the arena and presented with two identical objects, A1 and A2 during 5 min period (half of the animals were presented two black metal boxes and the other half two green glass cones). After a 60 min interval in the home cage, rats were again placed for 5 min in the same plastic box as earlier and presented with two objects: the old familiar A1, and a new object B (recognition session). Object A2 was always the one that was replaced. Object exploration was defined as