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Original article

Risperidone and escitalopram co-administration: A potential treatment of schizophrenia symptoms with less side effects

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ABSTRACT

Background: Schizophrenia is a psychiatric disorder characterized by positive and negative symptoms often accompanied by depression and cognitive deficits. Positive symptoms, like delusions and hallucinations are caused by an excess of dopamine (DA) signaling and are treated with the second generation antipsychotic drugs. Negative symptoms of schizophrenia are represented by social withdrawal, apathy and blunted emotional response. It was demonstrated that co-administration of risperidone and selective serotonin reuptake inhibitors alleviated depressive symptoms and cognitive dysfunction in animal models of schizophrenia. Moreover, combination of fluoxetine or mirtazapine with risperidone increased DA and 5-hydroxytryptamine (5-HT) release in the rat frontal cortex more potently than either drug given separately. The present study aimed to investigate whether combination of risperidone and escitalopram is effective in increasing DA and 5-HT release.

Methods: The extracellular level of neurotransmitters in the rat frontal cortex and nucleus accumbens was examined using microdialysis in freely moving animals. The dialysate concentration of DA and 5-HT was assayed by HPLC.

Results: It was found that risperidone (0.2 and 1 mg/kg) and escitalopram (5 and 10 mg/kg) given together significantly increased cortical DA and 5-HT levels and were more efficient in enhancing neurotransmitter concentrations than any single-drug treatment. A similar effect on DA and 5-HT release was observed in the nucleus accumbens after administration of risperidone (1 mg/kg) and escitalopram (5 mg/kg).

Conclusions: The present study demonstrates that co-administration of risperidone and escitalopram may be used to treat positive and negative symptoms of schizophrenia and will allow to minimize the drugs' side effects.

Introduction

Schizophrenia is a debilitating psychiatric disorder typically characterized by overlapping symptoms of psychosis, depression and cognitive impairment. Its etiology is still not well understood but dysregulation of monoaminergic transmission is potentially involved in clinical symptoms, such as delusions, hallucinations (so-called positive symptoms), amotivation, social withdrawal (negative symptoms) or cognitive deficits [1]. Positive symptoms of schizophrenia are due to an excess of dopamine (DA) signaling and recently are treated with second generation antipsychotics, while selective serotonin reuptake inhibitors (SSRI) are used as an adjunct to treat depression and cognitive symptoms [2,3]. Recently, it is also known that most of atypical antipsychotic drugs mediate their effects *via* weak antagonism at dopamine D2 receptors and strong antagonism at serotonin (5-HT) 2A receptors. Other 5-HT receptor subtypes, such as 5-HT2C, 5-HT6 and 5-HT7 may also contribute to the antipsychotic effect [4]. Risperidone is a second generation antipsychotic drug that at low doses blocks serotonin 5-HT2A receptors while at higher inhibits dopamine D2 receptors [5]. High 5-HT2A/D2 ratio after risperidone indicates a low risk of extrapyramidal symptoms with this drug. Beside the antipsychotic mechanism, the blockade of 5-HT2A receptors in cortical regions and inhibition of glutamatergic neurotransmission [6] implies also an antihallucinogenic effect of this drug [7]. Risperidone was reported to be an efficient antidepressant at doses of 0.5–1 mg/day in contrast to the doses of 3–6 mg/day used in schizophrenia [8]. Adjunct treatment with the SSRI escitalopram

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during risperidone administration may ameliorate depressive symptoms and cognitive dysfunction in schizophrenia. The bell-shaped dose-response curve of risperidone, with higher doses being less effective than lower doses, is consistent with the hypothesis that excessive D2 receptor antagonism may diminish the effects of 5-HT2A receptor blockade [9,10]. It was demonstrated that escitalopram co-administrated with an ineffective dose of risperidone abolished the deficit of object recognition memory induced by MK-801 in mice [11]. Antipsychotic-like effect of low dose of risperidone demonstrated by using the conditioned avoidance response in rats was dramatically enhanced by escitalopram, without increasing catalepsy [12]. In another study, using the social interaction test in rats that can model negative symptoms of schizophrenia, it was shown that escitalopram increased antipsychotic effect of an ineffective dose of risperidone [13]. Neurochemical data indicate that addition of some antidepressant drugs, such as mirtazapine, fluoxetine or citalopram can augment the monoamine release in rat brain regions induced by an antipsychotic drug [12,14,15,16,17].

The present study was undertaken to determine the efficacy of different doses of the novel selective serotonin re-uptake inhibitor, escitalopram as adjunct treatment to risperidone on DA and 5-HT release in the rat frontal cortex and the nucleus accumbens by microdialysis in freely moving animals.

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- and humidity- controlled rooms with a 12-h light-dark cycle (the light on at 7a.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).

Drugs administration

Animals were administered single intraperitoneal (*ip*) injections of risperidone (RIS, Tocris Bioscence, Bristol, UK) at a dose of 0.2 and 1 mg/kg and escitalopram oxalate (ESC, Tocris, Bristol, UK) at doses of 5 and 10 mg/kg. ESC was dissolved in a 0.9% NaCl while RIS was dissolved in 0.1 M tartaric acid solution and was adjusted to pH 6–7 with 0.1 M NaOH. Control animals received 0.9% NaCl or 0.1 M tartaric acid solution adjusted to pH 6–7 with 0.1 M NaOH. Both of the drugs were given as indicated in figures. All the chemicals used for high performance liquid chromatography (HPLC) were from Merck (Warszawa, Poland).

Microdialysis

Rats were anesthetized with ketamine (75 mg/kg *im*) and xylazine (10 mg/kg *im*), placed in a stereotactic apparatus (David Kopf Instruments, Tujunga, CA, USA) and subsequently microdialysis probes (2 and 3 mm, AgnAtho's, Sweden) were implanted in the rat frontal cortex or nucleus accumbens with the following coordinates (mm) A + 2.8, L + 0.8, V − 6.0 and A + 1.2, L + 1.0, V − 8.0 from the dura, respectively [18]. Twenty four hours after implantation, probe inlets were connected to a syringe pump (CMA, Sweden) which delivered an artificial CSF (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. Baseline samples were collected every 20 min after the washout period. 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. In the whole study, three rats were excluded due to probe misplacement. Each experimental group consisted of 6–7 rats.

Analytical procedure

DA and 5-HT were analyzed by HPLC with coulochemical detection. Chromatography was performed using the 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 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 were E1 = −50 mV, E2 = +300 mV and a sensitivity was set at 50 nA/V. The chromatographic data were processed by Chromeleon version 6.80 (Dionex, USA) software run on a PC computer. The limit of detection of DA and 5-HT in dialysates was 0.02 pg/10 μl for DA and 0.016 pg/10 μl for 5-HT.

Statistical analysis

The data were presented as the mean ± SEM. Average baseline values for all experiments were calculated from four samples prior to control, escitalopram, risperidone or their combination injection. The statistical significance was calculated using a repeated-measures ANOVA or where appropriate a one-way ANOVA. *Post-hoc* Tukey's test was used to analyze any significant treatments at specific time points. The results were considered statistically significant when $p < 0.05$.

Results

The effect of escitalopram or risperidone on DA and 5-HT release in the rat frontal cortex

Escitalopram 5 and 10 mg/kg in a dose-dependent manner increased the cortical extracellular level of DA and 5-HT reaching ca. 250% and 650% of the basal level, respectively, at the higher dose (Fig. 1A and C). There was a significant effect of treatment with the drug on DA [$F(2,14) = 91$, $p < 0.001$], 5-HT [$F(2,16) = 422$, $p < 0.001$]. There was the effect of time [$F(8112) = 11.2$, $p < 0.001$] for DA, [$F(8128) = 58$, $p < 0.001$] for 5-HT and significant interaction between both factors [$F(16,112) = 4.4$, $p < 0.001$] for DA, and [$F(16,128) = 10.6$, $p < 0.001$] for 5-HT.

Risperidone at doses 0.2 and 1 mg/kg increased the extracellular level of DA and 5-HT to ca. 250–350% of the basal level at the higher dose (Fig. 1B and D). There was a significant effect of treatment with the drug on DA [$F(2,14) = 813$, $p < 0.001$], 5-HT [$F(2,15) = 887$, $p < 0.001$]. There was the effect of time [$F(8112) = 40$, $p < 0.001$] for DA, [$F(8120) = 39$, $p < 0.001$] for 5-HT, and there was a significant interaction between both factors [$F(16,112) = 19$, $p < 0.001$] for DA, and [$F(16,120) = 29$, $p < 0.001$] for 5-HT.

The effect of escitalopram and risperidone co-administration on DA release in the rat frontal cortex

The levels of DA after treatment with the combination of escitalopram 5 mg/kg and risperidone 0.2 mg/kg were higher than when drugs were given separately (Fig. 2A). There was a significant effect of treatment [$F(3,19) = 216$, $p < 0.001$], significant effect of time [$F(8152) = 34$, $p < 0.001$] and significant interaction between both factors [$F(24,152) = 6.4$, $p < 0.001$]. *Post hoc* Tukey's test showed a significant difference between co-administration of