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Effect of risperidone on the fluoxetine-induced changes in extracellular dopamine, serotonin and noradrenaline in the rat frontal cortex

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Abstract:
Background: Several clinical reports have documented a beneficial effect of the addition of a low dose of risperidone to the ongoing treatment with antidepressants, in particular selective serotonin reuptake inhibitors, in the treatment of drug resistant depression. The aim of our study was to understand the mechanism of the clinical efficacy of a combination of fluoxetine (FLU) and risperidone (RIS) in drug-resistant depression. We studied the effect of FLU and RIS, given separately or jointly on the extracellular levels of dopamine (DA), serotonin (5-HT) and noradrenaline (NA) in the rat frontal cortex.
Methods: Animals were given single intraperitoneal injections of RIS at a doses of 0.1 or 1 mg/kg and FLU at a dose of 10 mg/kg. The release of DA, 5-HT and NA in the rat frontal cortex was investigated using microdialysis in freely moving animals. The extracellular level of DA, 5-HT and NA was assayed by HPLC with coulochemical detection.
Results: RIS (0.1 and 1 mg/kg) and FLU (10 mg/kg) increased the extracellular level of cortical DA, 5-HT and NA. Co-treatment of both drugs was more effective in increasing DA release than administration of each of the drugs alone at doses of RIS 1 mg/kg and FLU 10 mg/kg. Co-treatment of FLU and RIS 0.1 mg/kg was more potent than FLU alone, while the effect of joint injection of FLU and RIS 1 mg/kg was stronger than RIS 1 mg/kg alone on 5-HT release. The combination of FLU with both doses of RIS was not effective in increasing NA release as compared to drugs given alone.
Conclusions: Our data indicate that the effect of the combined administration of RIS and FLU on DA and 5-HT release in the rat frontal cortex may be of crucial importance to the pharmacotherapy of drug resistant depression.

Key words:
fluoxetine, risperidone, dopamine, serotonin or noradrenaline release, rats

Introduction

Up to 10% of the human population suffers from major depression. Although initial antidepressant (AD) therapy significantly reduces symptoms of depression in many patients, only 50–60% of persons with a major depressive disorder respond to the treatment. Moreover, ca. 30–40% of patients suffering from the major depressive disorder never achieve symptom resolution by means of a standard AD therapy [1, 48]. The problem of AD-resistant depression has been the subject of a number of thorough studies, with no apparent therapeutic success, though. Hence, there is a strong need for an alternative antidepressive treatment. Among the agents that are expected to potentiate the efficacy of ADs are atypical antypsychotics (e.g., aripiprazole, olanzapine, quetiapine, risperidone

(RIS), ziprasidone) [49, 57]. Several clinical reports have postulated a beneficial effect of an additional low dose RIS to ongoing treatment with ADs (in particular, selective serotonin reuptake inhibitors (SSRI), such as fluoxetine (FLU), fluvoxamine or paroxetine [18, 23, 32, 33, 38]. Like other atypical antipsychotic drugs, RIS is known to produce minimal extrapyramidal side-effects compared to classical antipsychotics (e.g., chlorpromazine) [3, 26, 31]. This drug is ca. 20–50 times more potent in its binding to 5-HT_{2A} serotonin receptors than to α_1-adrenergic, dopamine D_2, histamine H_1 and α_2-adrenergic receptors [39, 47]. It has been proposed that RIS in lower doses acts mainly by blocking 5-HT_{2A} serotonin receptors, while at higher doses it mostly blocks D_2 dopamine receptors. Our previous studies indicated that RIS applied at a low dose enhanced the antidepressant-like activity of ADs in the forced swimming test in animals [40, 42, 43].

Moreover, disturbances in dopamine (DA), serotonin (5-HT) and noradrenaline (NA) neurotransmitter systems have been suggested to be involved in the pathogenesis of mood disorders including depression [29, 46, 56].

A number of previous studies have shown that RIS (1 mg/kg), like other atypical antipsychotics (olanzapine, 3 mg/kg or clozapine, 3 mg/kg) varyingly increased the extracellular levels of both DA and NA in rat cortical areas, while RIS did not produce any changes in 5-HT in the frontal cortex, but increased it in the rat medial frontal cortex [17, 20]. FLU (10 mg/kg) robustly increased the extracellular levels of DA, NA and 5-HT in several brain regions, such as the striatum, hypothalamus and prefrontal cortex [34, 35]. Combination of RIS with FLU produced a significant increase in DA level, however, the effect on NA and 5-HT was not significantly different from the effect of FLU alone [59].

To understand the mechanism of the clinical efficacy of the AD and RIS combination therapy for treatment-resistant depression, the present study was designed to determine the influence of FLU (10 mg/kg) and RIS at two doses (0.1 and 1 mg/kg), given separately or jointly, on the extracellular levels of DA, 5-HT and NA in the rat frontal cortex of freely moving rats using microdialysis. The effect of co-treatment with a lower dose of RIS (0.1 mg/kg) and FLU, on the extracellular levels of monoamines in the rat frontal cortex, quantified using microdialysis has not been studied, yet.

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 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 at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Drugs and treatments

Animals were administered single intraperitoneal (*ip*) injections of risperidone (RIS, Tocris, Bristol, UK) at a doses of 0.1 or 1 mg/kg and FLU at a dose of 10 mg/kg (Pliva, Kraków, Poland). FLU was dissolved in 0.9% NaCl and RIS was dissolved in 0.1 M tartaric acid solution and was 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 a microdialysis probes 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 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 30 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.

Analytical procedure

DA, 5-HT and NA 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 5014B microdialysis cell and a Hypersil Gold-C18 analytical column (3 × 100 mm). The mobile phase was composed of 0.05 M potassium phosphate buffer adjusted to pH = 3.6, 0.5 mM EDTA, 16 mg/l 1-octanesulfonic acid sodium salt, and a 2.1% 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 PC computer.

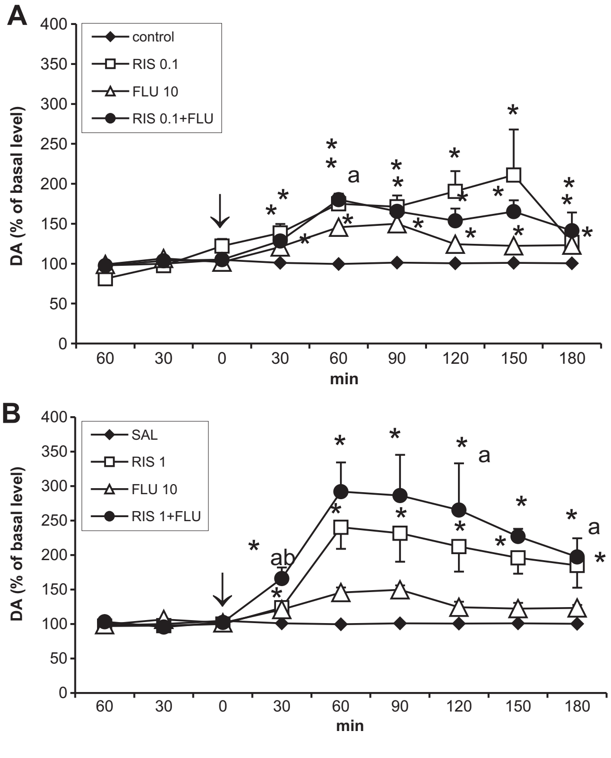

Fig. 1. The effect of risperidone (RIS 0.1 mg/kg, **A** or RIS 1 mg/kg, **B**) and fluoxetine (FLU 10 mg/kg, **A**, **B**) on the dopamine (DA) release in the rat frontal cortex. Drugs were given *ip* as indicated with an arrow. Each value is the mean ± SEM of 6 measurements and is expressed as a % of the basal level. The basal extracellular levels of DA (pg/10 µl) in animals treated with saline (SAL), RIS 0.1, RIS 1, FLU 10, RIS 0.1 + FLU, RIS 1 + FLU group were: 4.82 ± 0.49, 4.07 ± 0.14, 4.71 ± 0.74, 4.54 ± 0.27, 5.29 ± 0.65, 4.77 ± 0.94, respectively. * $p < 0.05$ *vs.* saline , SAL group; [a] $p < 0.01$ FLU + RIS *vs.* RIS group; [b] $p < 0.01$ FLU + RIS *vs.* FLU group (repeated measures ANOVA and Tukey's *post-hoc* test)

Data analysis

The statistical significance was calculated using repeated-measures ANOVA, followed by Tukey's *post-hoc* test. The results were considered statistically significant when $p < 0.05$.

Results

The effect of a single administration of RIS and FLU on DA release in the rat frontal cortex

There were no significant differences in the basal extracellular DA levels in the rat frontal cortex between the various treatment groups.

RIS (0.1 and 1 mg/kg,) significantly ($p < 0.01$) increased in a dose-dependent manner the extracellular level of cortical DA as compared to the control group. The increase in the extracellular DA level induced by FLU (10 mg/ kg) was weaker than that evoked by either dose of RIS. The combination of FLU and the higher dose of RIS (1 mg/kg) produced a stronger effect ($p < 0.01$) on the extracellular level of DA than FLU alone did (Fig. 1A, B).

The effect of a single administration of RIS and FLU on 5-HT release in the rat frontal cortex

There were no significant differences in the basal extracellular 5-HT levels in the rat frontal cortex between the various treatment groups.

RIS (0.1 or 1 mg/kg) and FLU (10 mg/kg) significantly increased the extracellular level of cortical 5-HT as compared to SAL group ($p < 0.01$). The effect of FLU and RIS 0.1 was weaker than FLU alone ($p < 0.01$), while the combination of FLU with RIS (1 mg/kg) was more potent than RIS (1 mg/kg) alone ($p < 0.01$) in influencing cortical 5-HT (Fig. 2A, B).

The effect of a single administration of RIS and FLU on NA release in the rat frontal cortex

There were no significant differences in the basal extracellular NA levels in rat frontal cortex between the various treatment groups.

RIS (0.1 and 1 mg/kg) and FLU (10 mg/kg) given separately elevated the extracellular level of NA as com-

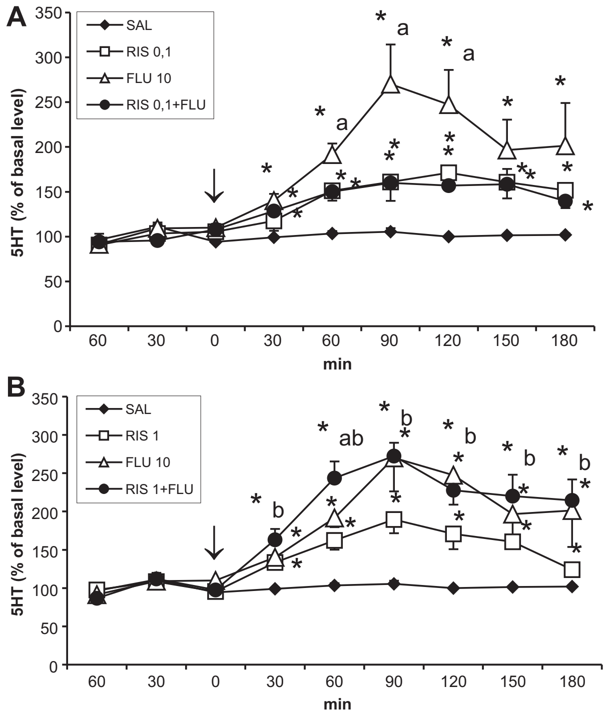

Fig. 2. The effect of risperidone (RIS 0.1 mg/kg, **A** or RIS 1 mg/kg, **B**) and fluoxetine (FLU 10 mg/kg, **A**, **B**) on the serotonin (5-HT) release in the rat frontal cortex. Drugs were given as indicated with an arrow. Each value is the mean ± SEM of 6 measurements and is expressed as a % of the basal level. The basal extracellular levels of 5-HT (pg/10 µl) in animals treated with saline (SAL), RIS 0.1, RIS 1, FLU 10, RIS 0.1 + FLU, RIS 1 + FLU group were: 1.58 ± 0.15, 2.34 ± 0.86, 1.28 ± 0.22, 1.68 ± 0.37, 1.67 ± 0.17, 1.61 ± 0.29, respectively. * $p < 0.05$ *vs.* saline, SAL group; [a] $p < 0.01$ FLU + RIS *vs.* RIS group; [b] $p < 0.01$ FLU + RIS *vs.* FLU group (repeated measures ANOVA and Tukey's *post-hoc* test)

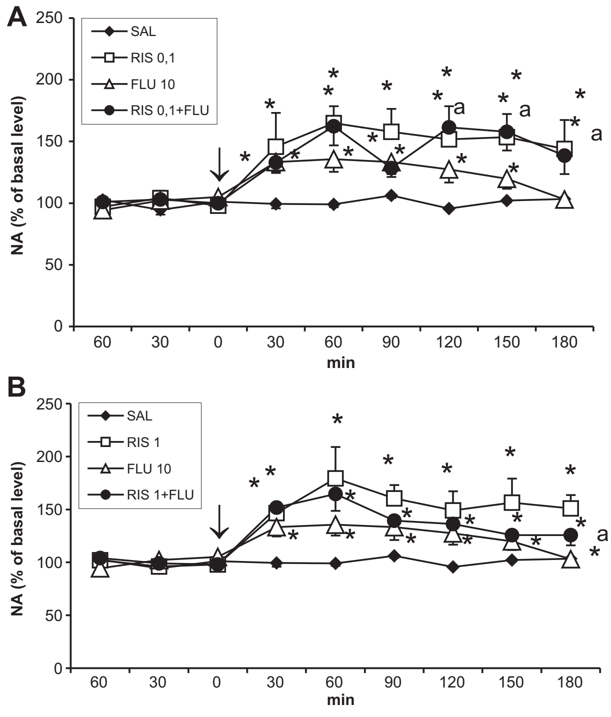

Fig. 3. The effect of risperidone (RIS 0.1 mg/kg, **A** or RIS 1 mg/kg, **B**) and fluoxetine (FLU 10 mg/kg, **A**, **B**) on the noradrenaline (NA) release in the rat frontal cortex. Drugs were given as indicated with an arrow. Each value is the mean ± SEM of 6 measurements and is expressed as a % of the basal level. The basal extracellular levels of NA (pg/10 µl) in animals treated with saline (SAL), RIS 0.1, RIS 1, FLU 10, RIS 0.1 + FLU, RIS 1 + FLU group were: 9.99 ± 0.91, 8.11 ± 0.87, 9.62 ± 0.93, 8.33 ± 0.42, 11.48 ± 1.27, 10.93 ± 1.3, respectively. * $p < 0.05$ *vs.* saline, SAL group; [a] $p < 0.01$ FLU + RIS *vs.* RIS group; (repeated measures ANOVA and Tukey's *post-hoc* test)

pared to the control group ($p < 0.01$), but the effect of co-treatment with both those drugs was not stronger than that after their separate administration (Fig. 3A, B).

Discussion

In our present study we investigated the effect of RIS at two doses (0.1 or 1 mg/kg), and FLU (10 mg/kg) given separately or jointly, on the extracellular levels of DA, 5-HT and NA in the rat frontal cortex of freely moving rats using microdialysis. RIS significantly increased in a dose-dependent manner the extracellular level of cortical DA, 5-HT and NA. Consistent with previous findings [16, 27, 28, 55, 59] atypical antipsychotic agents including olanzapine (3 mg/kg), clozapine (3 mg/kg) and RIS (1 mg/kg) increased both the extracellular DA and NA levels in rat cortical areas. Olanzapine, clozapine and RIS did not induce appreciable changes in 5-HT in the prefrontal cortex, although RIS was reported to increase 5-HT in the rat medial prefrontal cortex [17, 20]. Haloperidol, a typical antipsychotic with mainly D2/D3/D4 antagonistic properties [4] and MDL 100907, a selective 5-HT_{2A} antagonist [50] did not significantly change any of these monoamine levels.

The present study has shown that FLU significantly increased DA release, but its effect was weaker than that induced by either doses of RIS. Moreover, FLU significantly increased the extracellular level of cortical 5-HT and NA. Our earlier study showed that FLU significantly increased DA release without change in the extracellular DOPAC and HVA level in the rat frontal cortex. We also found that FLU increased the extracellular 5-HT level in the rat frontal cortex. However, the effect of FLU was not statistically significant when the whole 5-HT curve was compared to the control group, which indicates that FLU effect on 5-HT release in the rat frontal cortex is rather short-lasting, and limited to the first hour of drug action. On the other hand, FLU significantly and systematically decreased the extracellular 5-HIAA levels, as the response to the 5-HT reuptake inhibition causing disturbance in intraneuronal metabolism of 5-HT [41].

A number of previous studies showed an elevation of the extracellular DA and 5-HT concentrations in some brain regions, such as the frontal cortex, striatum and hypothalamus following FLU administration [11, 34, 53]. Moreover, it was shown that FLU treatment elevated the extracellular levels of NA in the prefrontal cortex [35, 59] and FLU, like citalopram (SSRI), increased 5-HT concentration [5, 19, 24, 58]. The effect of SSRI on DA release may be indirect, for instance, mediated by an increase in endogenous 5HT which was shown elsewhere [21, 30]. However, citalopram, unlike FLU [22, 54] did not increase the extracellular DA and NA level in rats prefrontal cortex, as previously reported by Bymaster et al. [5] and Koch et al. [25]. This suggests that the increased DA and NA efflux induced by FLU alone is unlikely to be secondary to its effect on 5-HT efflux since citalopram, the most selective SSRI [45], produces a greater increase in 5-HT efflux than FLU does [5, 12], but has no effect on the extracellular DA and NA concentrations. Moreover, local infusion of citalopram at 0.1–10 μM into the prefrontal cortex markedly increased the extracellular 5-HT, but had little or no effect on DA efflux, clearly showing that the extracellular 5-HT concentrations can be substantially increased by SSRIs in the frontal cortex without any concomitant changes in the extracellular DA [37]. On the other hand, evidence has been obtained that inhibition of NA uptake in the frontal cortex enhances the extracellular concentrations of DA as well as NA [6, 36], providing a possible mechanism of the effect of FLU to enhance DA efflux [37].

Co-treatment with FLU and a higher dose of RIS produced a stronger effect on the extracellular level of cortical DA than FLU alone. We also found that co-treatment with FLU and RIS at a lower dose (0.1 mg/kg) was more potent than FLU alone, while a combination of FLU with RIS at a higher dose (1 mg/kg) was more potent than RIS alone. FLU and RIS elevated the extracellular levels of NA, but the effect of co-treatment with both those drugs was not stronger than these drugs caused given alone. The previous study indicated that RIS at a dose of 1 mg/kg significantly increased DA, 5-HT and NA in the rat medial prefrontal cortex [20]. Citalopram (10 mg/kg) induced a significant increase in 5-HT levels only, but co-treatment with RIS and citalopram evoked significantly greater increases in efflux of both DA and NA compared to RIS alone. However, the effect of this combination on the extracellular 5-HT concentrations was not significantly different than that of citalopram alone. The increase in DA and NA efflux induced by RIS and citalopram could be partially blocked by the selective 5-HT_{1A} antagonist, WAY 100635, which suggests contribution of 5-HT_{1A} receptor stimulation in the mechanism of action of both drugs [19].

The present biochemical data are in line with our behavioral observation which showed that RIS at a low dose (0.05 or 0.1 mg/kg) potentiated the antidepressant-like activity of FLU (10 mg/kg) or mirtazapine (5 mg/kg) in the forced swimming test in rats, and the selective 5-HT_{1A} antagonist, WAY 100635 (0.1 mg/kg) abolished that effect [43].

It is postulated further that 5-HT_{2A} receptors play an important role in mediating serotonin action on the extracellular level of cortical DA. For instance, RIS is about 20–50 times more potent in binding to 5-HT_{2A} receptors than to α_1-adrenergic, dopamine D_2-, and α_2-adrenergic ones, and also shows a slight affinity for histamine H_1 receptors [39, 47]. It is suggested that the selectivity of RIS for 5-HT_{2A} *vs.* 5-HT_{2C} receptors offers a more favorable therapeutic option in various mood disorders including depression. However, the addition of RIS to serotonergic ADs may trigger complex interactions between the serotonergic, dopaminergic and/or noradrenergic systems. It has been postulated that administration of SSRIs leads to a decrease in norepinephrine neuronal firing [44], and subsequently, builds up resistance to its antidepressant action, which can be overcome by administration of RIS, a 5-HT_{2A} receptors antagonist. RIS is known to reverse the SSRI-induced inhibition of the activity of norepinephrine neurons by a mechanism involving 5-HT_{2A} receptors [10]. Hence, the drugs that exert both those effects (5-HT reuptake inhibition and 5-HT_{2A} receptor antagonism) may have a more beneficial therapeutic action compared to SSRIs alone.

Moreover, adrenergic α_2 receptor blockade may also contribute to the ability of the RIS and FLU co-administration to increase DA efflux in the frontal cortex. Adrenergic α_2 receptors located in both the dendrites and terminals of frontocortical adrenergic pathways exert a pronounced tonic, inhibitory influence to the efflux DA and NA in the frontal cortex [13]. It was shown that buspirone, by activation of 5-HT_{1A} and blockade of α_2-adrenergic receptors, elevated FLU-stimulated dialysate levels of DA and NA, but not 5-HT, in the frontal cortex [14]. In addition, idazoxan, an adrenergic-α_2 receptor antagonist, enhanced the effect of the D_2 receptor antagonist, raclopride to increase DA efflux in the medial prefrontal cortex [15]. Furthermore, earlier behavioral studies indicated that yohimbine, an α_2-adrenergic receptor antagonist, enhanced the antidepressant-like action of venlafaxine and FLU and potentiated the effect of joint treatment with those antidepressants and RIS in the forced swimming test in mice, which suggested a role of α_2-adrenoreceptors in those effects [9, 40].

It is known that FLU is metabolized primarily *via* *N*-demethylation to the active metabolite norfluoxetine. The results of *in vivo* and *in vitro* studies have indicated that CYP2D6 is the major isoenzyme responsible for *N*-demethylation of FLU with additional contributions from CYP2C9, CYP2C19 and CYP3A4. *In vitro* studies have found that FLU and norfluoxetine are potent inhibitors of CYP2D6 and moderate inhibitors of CYP2C9, whereas they have a mild to moderate effect on the activity of CYP2C19 and CYP3A4. Therefore, FLU has a high potential for clinically relevant pharmacokinetic interactions with other agents. FLU and its active metabolite have a long $t_{1/2}$ (7–14 days), and CYP inhibition may continue for weeks after the treatment discontinuation [7, 8, 52]. A clinically relevant pharmacokinetic interaction may also occur between FLU and RIS [2, 52]. In an open-label study in 10 schizophrenic patients co-treatment with RIS (4–6 mg/day) and FLU (20 mg/kg) for 4 weeks was associated with a 75% elevation in plasma concentration of the active fraction of RIS [51]. This interaction was assumed to be the result of inhibition of CYP2D6, the major isozyme responsible for the 9-hydroxylation of RIS, although it is possible that norfluoxetine also inhibited CYP3A4, blocking both metabolic pathways for RIS. A reduction in the RIS dose is advisable when this agent is co-administered with FLU [51]. Interestingly, augmentation of FLU (20 mg/day) with RIS at lower doses (0.5–3 mg/day) for patients with difficult to treat depression leads to a more rapid response, higher remission rate and better quality of life, also there were no differences in the number of adverse events between groups (patients in RIS group and patients in the placebo group), which indicates the lack of a pharmacokinetic interaction [23].

In summary, the present study shows for the first time that RIS used at a lower dose (0.1 mg/kg) increases the extracellular levels of cortical DA, 5-HT and NA. Moreover, co-treatment with FLU (10 mg/kg) and RIS (0.1 mg/kg) was more potently increased DA release than does FLU alone (but not 5-HT or NA). Our present data are also in line with a previous study which showed that RIS at a higher dose (1 mg/kg) and FLU (10 mg/kg) increased the extracellular levels of cortical DA, 5-HT and NA, and that a combination of RIS (1 mg/kg) and FLU (10 mg/kg) significantly increased the extracellular level of both DA and 5-HT (but not NA). The above findings suggest that the increase in the extracellular levels of DA and 5-HT may play some role in the enhancement of FLU efficacy by RIS, and may be of crucial importance to the pharmacotherapy of drug-resistant depression. Further studies are necessary to elucidate its mechanism of action, especially after repeated administration of RIS (both in the lower and the higher dose) and FLU.

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