



Effects of co-administration of fluoxetine or tianeptine with metyrapone on immobility time and plasma corticosterone concentration in rats subjected to the forced swim test

Zofia Rogóż¹, Grażyna Skuza¹, Monika Leśkiewicz²,
Bogusława Budziszewska²

¹Department of Pharmacology, ²Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Zofia Rogóż, e-mail: rogoz@if-pan.krakow.pl

Abstract:

Major depression is frequently associated with hyperactivity of the hypothalamic-pituitary-adrenocortical axis, and glucocorticoid synthesis inhibitors have been shown to exert antidepressant action. The aim of the present study was to examine the effect of co-administration of fluoxetine or tianeptine with metyrapone on immobility time and plasma corticosterone concentration in male Wistar rats subjected to the forced swim test. Metyrapone alone (50 mg/kg, but not 25 mg/kg) reduced the immobility time of rats in the forced swim test; moreover, both doses tested (25 and 50 mg/kg), dose-dependently decreased the stress-induced plasma corticosterone concentration. Joint administration of fluoxetine or tianeptine (10 mg/kg) and metyrapone (25 mg/kg – a dose inactive *per se*) exhibited antidepressant-like activity in the forced swim test in rats. WAY 100636 (a 5-HT_{1A} antagonist), but not prazosin (an α_1 -adrenergic antagonist), used in doses ineffective in the forced swim test, inhibited the antidepressant-like effect induced by co-administration of fluoxetine or tianeptine with metyrapone (25 mg/kg). Combined treatment of fluoxetine or tianeptine and metyrapone inhibited stress-induced corticosterone secretion to a similar extent as metyrapone alone. The obtained results indicate that metyrapone potentiates the antidepressant-like activity of fluoxetine or tianeptine and that, among other mechanisms, 5-HT_{1A} receptors may play some role in this effect. Moreover, metyrapone exerts a beneficial effect on the stress-induced increase in plasma corticosterone concentration. These findings suggest that the co-administration of metyrapone and an antidepressant drug may be useful for the treatment of drug-resistant depression and/or depression associated with a high cortisol level.

Key words:

fluoxetine, tianeptine, metyrapone, forced swim test, corticosterone, rats

Introduction

Major depression is frequently associated with hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis. Clinical studies have shown that depressed patients have an increased concentration of

cortisol in the plasma and cerebrospinal fluid, as reflected by an abnormal 24-h pattern of cortisol and ACTH secretion and elevated levels of corticotropin-releasing hormone in the cerebrospinal fluid [18, 27, 32]. A high incidence of depression in Cushing's syndrome, as well as the antidepressant action of cortisol synthesis inhibitors and antagonists of corticotropin-

releasing hormone (CRH) receptors, suggests that the hyperactivity of HPA is involved in the pathogenesis of the above-mentioned disorder [21, 31, 34]. A large number of the obtained data indicate that the hyperactivity of the HPA axis in major depression may be induced *via* a decreased inhibitory feedback mechanism [15, 35]. In fact, the synthetic glucocorticoid dexamethasone is less potent in lowering cortisol levels (basal and that induced by CRH) in the blood of depressed patients than in that of healthy subjects [15, 16]. The dysfunction of the HPA axis is corrected during clinically effective therapy with antidepressant drugs, while the persistence of dexamethasone non-suppression is often associated with the risk of relapse or the lack of improvement [15, 17]. The currently used antidepressant drugs show a therapeutic efficacy in about 60–70% of depressed patients only (e.g., [24]). Therefore, to improve therapy, a combination of antidepressants belonging to various pharmacological groups or a combination of an antidepressant drug and a substance that can enhance its effect is used in the clinic (e.g., [3, 10, 28, 41, 42]). Among agents that are expected to potentiate the efficacy of antidepressants are inhibitors of glucocorticoid synthesis. In fact, they have already shown antidepressant-like properties in some animal models of depression [1, 14]. In addition, clinical studies have demonstrated some antidepressant effects of metyrapone, aminoglutethimide and ketoconazole; however, these drugs are used mainly in drug-resistant depression [31, 37, 38]. To date, in the clinic, glucocorticoid inhibitors or antagonists of glucocorticoid receptors have been administered alone in relatively high doses, so their side-effects are occasionally observed [37]. A combination of a glucocorticoid inhibitor and an antidepressant drug should help decrease their doses and, in consequence, their side-effects as well. Among glucocorticoid inhibitors, metyrapone (an inhibitor of the enzyme 11- β -hydroxylase) has the weakest effect on gonadal hormone levels [38]. We previously found that combined treatment with imipramine and metyrapone produced a more potent antidepressant-like effect than did either of the drugs given alone in the forced swimming test (FST) in rats [40, 43]. Additionally, other studies indicated that joint administration of antidepressants and metyrapone led to clinical improvement [19, 44]. The aim of present study was to examine the effect of joint administration of other antidepressants, fluoxetine (FLU), selective serotonin reuptake inhibitors (SSRI), or tianeptine (TIA), a se-

lective serotonin reuptake enhancer, and metyrapone on the immobility time of rats subjected to the forced FST, as well as on the plasma corticosterone concentration in those rats. Furthermore, we used 5-HT_{1A}- and α_1 -adrenergic receptor antagonists to determine the role of those receptors in the antidepressant-like effect induced by joint treatment with FLU or TIA and metyrapone in the FST.

Materials and Methods

Animals

Male Wistar rats (230–250 g), purchased from a licensed dealer (Górzowska, Warszawa, Poland), were kept under standard animal house conditions (a room temperature of 23°C and a 12/12 h light/dark cycle, with the light on at 07:00), with food and water provided *ad libitum*. The rats were randomly divided into eight groups and were acclimatized for at least one week before the experiment. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). All experimental protocols were approved by the Local Bioethics Commission for the Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Rat forced swim test

The animals were individually subjected to two trials during which they were forced to swim in a cylinder (40 cm high, 18 cm in diameter) filled with water (25°C) to a height of 15 cm. There was a 24-h interval between the first and the second trial. The first trial lasted 15 min, while the second one was carried out for 5 min. The total duration of immobility was measured throughout the second trial [36]. FLU or TIA (10 mg/kg) and metyrapone (25 or 50 mg/kg) were dissolved in distilled water; all drugs or distilled water (vehicle) were injected intraperitoneally (*ip*) at three times: at 24, 5 and 1 h before the test. FLU or TIA was also co-injected with metyrapone in doses and at times stated above. WAY 100635 (0.1 mg/kg) was given at 15 min, and prazosin (PRA, 1 mg/kg) at 30 min before metyrapone and FLU or TIA. Control rats were injected with the vehicle. Each group consisted of eight rats.

Exploratory activity of rats in the open field test

FLU or TIA (10 mg/kg) and metyrapone (25 or 50 mg/kg) were given three times, at 24, 5 and 1 h before the test (like in the FST). Exploratory activity was assessed in an elevated open field test using a method that was a slightly modified procedure of Janssen et al. [20]. A black circular platform without walls, 1 m in diameter, was divided into six symmetrical sectors and was elevated 50 cm above the floor. The laboratory room was dark and only the centre of the open field was illuminated with a 75 W bulb placed 75 cm above the platform. At the beginning of the test, the animals were gently placed in the centre of the platform and were allowed to explore their surroundings. Their exploratory activity in the open field, i.e., the time of walking, the number of sector line crossings (ambulations), episodes of peeping under the edge of the area and rearing, were assessed for 5 min. Each group consisted of eight rats.

Corticosterone concentration in rat plasma

The blood for corticosterone level determinations was collected following decapitation at 2 h after the last vehicle/drug injection and 1 h after the FST. Control groups were not subjected to the FST. The blood was collected in EDTA and was centrifuged at 800 g for 15 min, after which the supernatant was removed and stored at -20°C until analysis. Corticosterone was extracted from the plasma to ethanol and was measured by a radioimmunological method. Ethanol plasma extracts were dried under a nitrogen stream, dissolved in 0.1 ml of 0.05 mM phosphate buffer, pH = 7.0, containing a 0.9% NaCl and a 0.1% gelatin (Sigma Chemical Co., USA), and were incubated with a 0.1 ml solution of 1,2,6,7- ^{3}H -corticosterone (20,000 dpm/sample; Amersham, s.a. 85 Ci/mmol) and with a 0.1 ml solution of a corticosterone antibody (Biogenesis) for 16 h at 4°C . Free and bound corticosterone was separated using a dextran-coated charcol. The samples were incubated for 10 min at 4°C with 0.2 ml of a 0.05% dextran (Dextran T 70, Pharmacia, USA) and 0.5% charcol (activated, Sigma, USA) suspension. After centrifugation at 1,000 g for 20 min, 0.3 ml of the supernatants was placed in a scintillator and radioactivity was measured with a β -counter (Beckmann LS 335). Corticosterone content was calculated using a log-logit transformation. The assay sensitivity was 10 pg/tube. Intra- and interassay coefficients of variation were

lower than 5 and 8%, respectively. Each group consisted of 6–8 rats.

Drugs

Fluoxetine hydrochloride (FLU, Farmacom, Poland), 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone, Aldrich, USA), prazosin hydrochloride (PRA, Research Biochemicals Inc., USA), tianeptine (TIA, Coaxil, Servier, France), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)-cyclo-hexanecarboxamide trihydrochloride (WAY 100635; synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were used for the present study.

Statistical analysis

The behavioral data were evaluated by a one-way analysis of variance (ANOVA) followed, when appropriate, by individual comparisons with the control using Dunnett's test. Statistical significance of the corticosterone level was assessed using Duncan's test.

Results

Rat forced swim test

Neither FLU (10 mg/kg) or TIA (10 mg/kg), nor metyrapone (25 mg/kg) given alone modified the immobility time of rats in the FST. At a higher dose (50 mg/kg), metyrapone exhibited antidepressant-like activity, shortening the immobility time of rats. Combined treatment with FLU (10 mg/kg) or TIA (10 mg/kg) and metyrapone (25 or 50 mg/kg) produced stronger inhibition of immobility than did either drug alone; moreover, the difference was statistically significant (Fig. 1, 2). WAY 100635 (0.1 mg/kg) was ineffective in the FST (data not shown), but inhibited the antidepressant-like effect induced by co-administration of FLU (10 mg/kg) and metyrapone (25 mg/kg) (Fig. 3), or TIA (10 mg/kg) and metyrapone (25 mg/kg) (Fig. 4). Moreover, PRA (1 mg/kg) modified neither immobility time (data not shown) nor the antidepressant-like effect induced by co-administration of FLU or TIA (10 mg/kg) and metyrapone (25 mg/kg) (Fig. 3, 4).

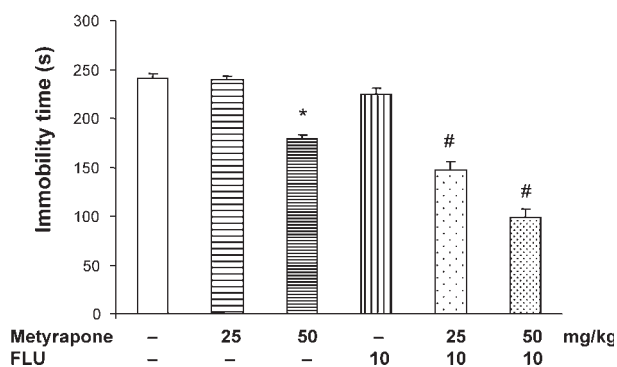


Fig. 1. The effect of metyrapone (25 or 50 mg/kg), given alone or in combination with fluoxetine (FLU, 10 mg/kg), on immobility time in the forced swim test in rats. Metyrapone and FLU were administered at three times (24, 5 and 1 h) before the test. The results are presented as the mean \pm SEM of eight animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.001$ vs. vehicle-treated group, # $p < 0.001$ vs. metyrapone- or FLU-treated group

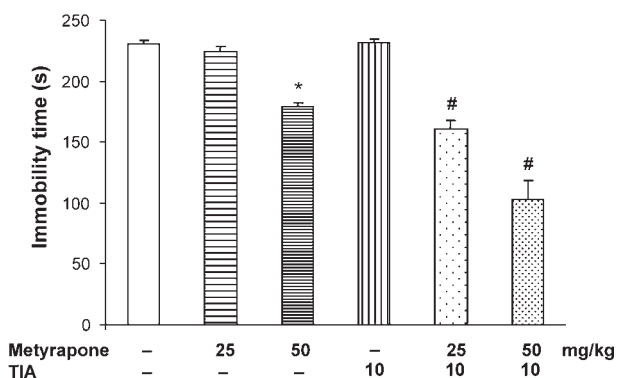


Fig. 2. The effect of metyrapone (25 or 50 mg/kg), given alone or in combination with tianeptine (TIA, 10 mg/kg), on immobility time in the forced swim test in rats. Metyrapone and TIA were administered at three times (24, 5 and 1 h) before the test. The results are presented as the mean \pm SEM of eight animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.001$ vs. vehicle-treated group, # $p < 0.001$ vs. metyrapone- or TIA-treated group

Exploratory activity of rats in the open field test

None of the tested drugs, i.e., neither FLU (10 mg/kg), TIA (10 mg/kg), metyrapone (25 or 50 mg/kg) – alone or in combination with FLU (10 mg/kg), nor TIA (10 mg/kg) and metyrapone (25 or 50 mg/kg), changed the exploratory activity of rats in the open field (data not shown). The 5-HT_{1A} receptor antago-

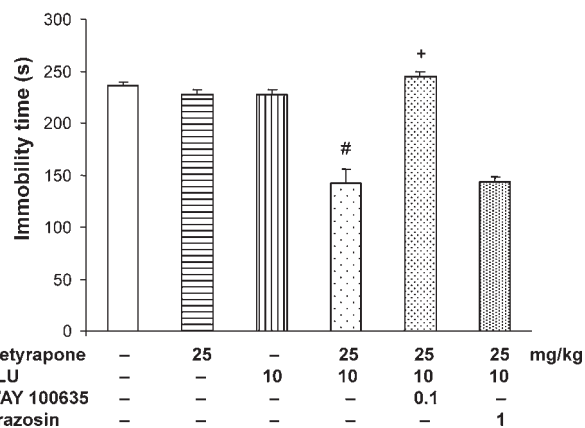


Fig. 3. The influence of WAY 100635 or prazosin (PRA) on the effect of combined treatment with metyrapone and fluoxetine (FLU) in the forced swim test in rats. Metyrapone and FLU were administered at three times (24, 5 and 1 h) before the test. WAY 100635 was given at 15 min, and PRA at 30 min before metyrapone and FLU. The results are presented as the mean \pm SEM of eight animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. # $p < 0.001$ vs. metyrapone- or FLU-treated group, + $p < 0.001$ vs. metyrapone + FLU-treated group

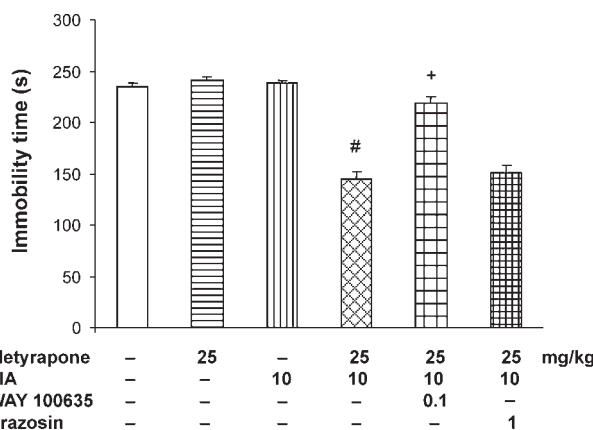
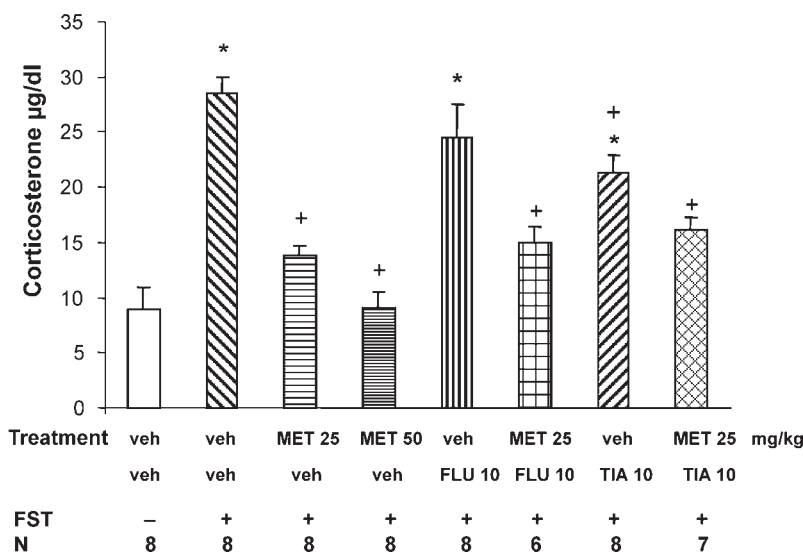


Fig. 4. The influence of WAY 100635 or prazosin (PRA) on the effect of combined treatment with metyrapone and tianeptine (TIA) in the forced swim test in rats. Metyrapone and TIA were administered at three times (24, 5 and 1 h) before the test. WAY 100635 was given at 15 min, and PRA at 30 min before metyrapone and TIA. The results are presented as the mean \pm SEM of eight animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. # $p < 0.001$ vs. metyrapone- or TIA-treated group, + $p < 0.001$ vs. metyrapone + FLU-treated group

nist WAY 100635 (0.1 mg/kg) and the α_1 -adrenergic receptor antagonist PRA (1 mg/kg) in doses used in the FST neither changed exploratory activity in a statistically significant manner, nor decreased the effect induced by joint administration of FLU or TIA and metyrapone (data not shown).

Fig. 5. The effect of exposure to the forced swim test (FST) and metyrapone, fluoxetine (FLU) or tianeptine (TIA) administration on plasma corticosterone concentration 1 h after the FST. Metyrapone, FLU or TIA was administered at three times (24, 5 and 1 h) before the test. The control group was not subjected to the FST. The results are presented as the mean \pm SEM (N = 6–8 animals/group). The data were statistically evaluated by ANOVA, followed by individual comparisons using Duncan's test. * $p < 0.001$ vs. vehicle (veh)/no-FST group, + $p < 0.001$ vs. veh-FST group



Corticosterone concentration in rat plasma

In the FST, plasma corticosterone level was significantly (ca. 3-fold) enhanced when assessed 1 h after the stress procedure (Fig. 5). In rats treated with metyrapone (25 and 50 mg/kg) and TIA (10 mg/kg) alone significantly attenuated the increase in plasma corticosterone concentration following FST exposure. Administration of FLU (10 mg/kg) alone had no significant effect on that corticosterone concentration; nevertheless, a tendency towards a decrease was observed. Joint treatment with metyrapone (25 mg/kg) and FLU (10 mg/kg) or TIA (10 mg/kg) decreased corticosterone level to a similar extent as did metyrapone (25 mg/kg) alone (in a statistically significant manner) compared to the group not treated with metyrapone.

Discussion

Our results show that, when used at a higher dose (50 mg/kg), metyrapone, a glucocorticoid synthesis inhibitor, reduces the immobility time of rats subjected to the FST procedure. These data are in line with our [33, 37] and other authors [1, 14, 23] earlier findings, which have revealed antidepressant-like properties of metyrapone in various animal models of depression (FST, olfactory bulbectomy and restraint stress paradigm). Metyrapone reduced immobility time with ef-

ficacy comparable to that of tricyclic antidepressants, e.g., desipramine [14] or imipramine [40, 44]. Moreover, our present study showed that joint administration of metyrapone (in both doses tested) and FLU or TIA reduced immobility time more potently than did either of the drugs given alone. In addition, WAY 100635 (a 5-HT_{1A} receptor antagonist) [12] inhibited the antidepressant-like effect induced by co-administration of FLU or TIA and metyrapone; on the other hand, PRA (a α_1 -adrenergic receptor antagonist) did not change the antidepressant-like effect induced by co-administration of FLU or TIA and metyrapone. We previously described a similar effect of metyrapone administered jointly with imipramine on immobility time in the FST in rats [43].

It is noteworthy that SSRI and TIA do not usually show antidepressant-like activity in FST in rats treated in accordance with the original method of Porsolt [36]. However, Detke and Lucki [8] and Detke et al. [9] have reported positive effects of SSRIs such as FLU or sertraline, or Molodavkin et al. [30] with FLU and TIA in rats, but using a modified forced swimming procedure, with extended scoring of immobility, swimming, climbing and diving. In general, the antidepressant effects of the SSRI appear after chronic treatment [7, 33]. Furthermore, TIA (10 mg/kg twice daily, for 14 days) significantly reduced both the expression of serotonin transporter mRNA and serotonin transporter binding sites labelled by [³H]paroxetine in the rat dorsal raphe nucleus. In the median raphe nucleus, TIA did not change either expression of sero-

tonin transporter mRNA or binding. This effect, which is similar to a reported effect for SSRI and imipramine, may help to explain the antidepressant effect of TIA [25, 26, 47], and suggest the possibility of a common action for these drugs in spite of their opposite effects on serotonin re-uptake.

In addition, our earlier findings indicated that repeated co-treatment with imipramine and metyrapone inhibited more potently 5-HT_{1A} neurotransmission in behavioral tests than did imipramine alone, and that neither acute nor chronic administration of metyrapone significantly altered that effect [39]. In contrast to the above data, the previously demonstrated decrease in reactivity of the 5-HT_{1A} system, observed after repeated treatment with desipramine and metyrapone in the behavioral experiment attenuated the hypothermic response to acute challenge with 8-OH-DPAT [14]. The ability to alter 8-OH-DPAT-induced hypothermia suggests that metyrapone and desipramine can alter 5-HT_{1A} receptor sensitivity, which has also been demonstrated for other ADs [13], and that 5-HT_{1A} receptor function is modulated by glucocorticoids. This concept is consistent with the observation that adrenalectomy can alter receptor binding characteristics and can increase 5-HT_{1A} receptor mRNA expression [4], with both of these effects being reversed by corticosterone supplementation. The above findings point to similarities between metyrapone and desipramine despite their different biochemical properties; moreover, they suggest that the serotonergic system may be involved in the mechanism underlying their antidepressant action.

Importantly, the reduced immobility time evoked by metyrapone may be a consequence of inhibition of stress-induced corticosterone secretion. Like other inescapable stress paradigms, the FST elevated blood corticosterone levels. In the model used in the present study in which animals were subjected to pre-testing one day prior to the experiment, one hour after the FST blood corticosterone concentrations were approximately three-fold higher than in control, non-stressed animals. Increases in corticosterone level depend on the kind and duration of stress, and are usually the highest shortly after single stress. It was found that the corticosterone plasma level rose about forty-fold immediately after a 20-min acute forced swim [22]. In line with our results, Baez and Volosin [1] showed that the FST procedure (the same one as that used in our study), evoked a 1.5- to 2-fold increase in corticosterone concentration 1 h after the

test. We found that systemic administration of metyrapone in a dose of 50 mg/kg (three times; at 24, 5 and 1 h before the test) not only reduced the immobility time of rats subjected to the FST procedure, but also suppressed the stress-induced rises in blood corticosterone level by approximately 50% [40]. The effect of metyrapone on immobility time has been the topic of several studies aimed at determining its action on plasma corticosterone levels in rats subjected to the FST procedure, but which had only been previously reported on by Baez and Volosin [1], in whose study metyrapone had been administered once at a higher dose (150 mg/kg). Despite the differences in metyrapone administration between the paper cited above and our study [40], this inhibitor decreased plasma corticosterone levels by about two-fold one hour after Porsolt's test. Moreover, Baez and Volosin [1] showed that the effect of metyrapone on immobility time and corticosterone level in the FST was reversed by corticosterone supplementation, which proved that the antidepressant-like action of metyrapone resulted from its effect on corticosterone level. Metyrapone, an 11- β -hydroxylase inhibitor, blocks the synthesis and subsequent release of corticosterone (in rats) or cortisol (in humans) to the bloodstream. It has been found that metyrapone suppresses plasma corticosterone concentration in stressed animals, but does not change the basal level of this steroid [45]. This finding seems to be particularly important, since in light of the corticosteroid receptor hypothesis of depression, reduction of the elevated, but not basal, glucocorticoid level is beneficial to the treatment of depression. Glucocorticoids act *via* two distinct receptors: a high-affinity mineralocorticoid receptor (MR) and a low-affinity glucocorticoid receptor (GR). MR is primarily involved in the regulation of basal glucocorticoids levels and function, while GR, activated by a high concentration of steroid are more important to the restoring of homeostasis after stress. The blockade of GR produces an antidepressant effect in experimental and clinical trials; however, due to the lack of a specific GR antagonist, and because of the adverse effects of mifepristone, this treatment strategy has not been studied in detail so far [16, 31, 37]. On the other hand, the MR antagonist spironolactone has been found to impair the response to antidepressant drugs [16]. Hence, the inhibitory effect of metyrapone on stress-induced corticosterone concentration only seems to be sufficient for the treatment of depression. In comparison with other glucocorticoid synthesis in-

hibitors, e.g., aminoglutethimide or ketoconazole, metyrapone acts more selectively on glucocorticoid synthesis and weakly affects gonadal steroid secretion [31, 38]. However, *via* inhibition of 11- β -hydroxylase, metyrapone increases the concentration of corticosteroid 11-deoxy precursors, including the positive modulator of the GABA_A receptor, tetrahydrodeoxycorticosterone [38]. Hence, the antidepressant effect of metyrapone seems to be connected not only with the reduction of plasma corticosterone concentration, but also with the action of bioactive corticosterone precursors on GABA-ergic transmission [29].

In the present study, TIA decreased corticosterone levels, and FLU in the dose tested also tended to decrease these levels, although the latter effect was statistically insignificant. It has been found that, in contrast to acute treatment, long-term administration of antidepressants decreases ACTH and corticosterone levels in the blood and CRH concentration in the hypothalamus [2, 17]. However, the effect of antidepressant drugs on HPA axis activity depends on the time of their administration, the type, time and duration of stress, as well as time on the mode of blood collection. In the case of the FST, sub-chronic administration of antidepressant drugs decreased stress-induced corticosterone concentrations in some experiments, but often no effect or only a tendency towards a decrease was observed [5, 6, 11, 46]. The above data indicate that three-fold administration of FLU or TIA and metyrapone shortened the immobility time more potently than did either of the drugs given alone. Joint administration of metyrapone and FLU or TIA inhibited corticosterone levels to a similar extent as did metyrapone alone. We have previously described a similar effect of metyrapone administered jointly with imipramine on corticosterone concentration and the reverse action of those drugs on immobility time [40]. The lack of effect of the synergistic action of metyrapone and antidepressants (e.g., FLU, TIA or imipramine) on corticosterone levels may be connected with the fact that antidepressant drugs are assumed to inhibit HPA axis activity by enhancing glucocorticoid feedback mechanisms, which are inhibited by metyrapone.

In conclusion, sub-chronic co-administration of FLU or TIA with metyrapone induces more potent antidepressive activity in the FST than does either of those drugs alone; moreover, in addition to their other actions, 5-HT_{1A} receptors may play some role in the antidepressant-like effects of metyrapone and FLU or

TIA in rats. Co-administration of antidepressant drugs and metyrapone reduces stress-induced corticosterone concentration, to a similar extent as metyrapone alone. Since joint administration of metyrapone and an antidepressant drug may help reduce their doses and, in consequence, also their side effects, the above reported findings may be helpful in choosing a proper drug combination for the treatment of drug-resistant depression and/or depression associated with a high cortisol level.

Acknowledgments:

The authors wish to thank Farmacom, Kraków (Poland) for their generous gift of fluoxetine. The authors would also like to thank Ms. E. Smolak, M.A., for the linguistic correction of the paper and Ms. B. Korzeniak for her skilful technical assistance.

References:

1. Baez M, Volosin M: Corticosterone influences forced swim-induced immobility. *Pharmacol Biochem Behav*, 1994, 49, 729–736.
2. Brady LS, Whitfield HJ, Fox RJ, Gold PW, Herkenham M: Long-term antidepressant administration alters corticotropin-releasing hormone, tyrosine hydroxylase, and mineralocorticoid receptor gene expression in rat brain. *J Clin Invest*, 1991, 87, 831–837.
3. Bschor T, Canata B, Muller-Oerlinghausen B, Bauer M: Predictors of response to lithium augmentation in tricyclic antidepressant-resistant depression. *J Affect Disord*, 2001, 64, 261–265.
4. Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ: Corticosteroids regulate brain hippocampal 5-HT_{1A} receptor mRNA expression. *Trends Neurosci*, 1993, 13, 914–923.
5. Connor TJ, Kelliher P, Shen Y, Harkin A, Kelly JP, Leonard BE: Effect of subchronic antidepressant treatments on behavioral, neurochemical, and endocrine changes in the forced-swim test. *Pharmacol Biochem Behav*, 2000, 65, 591–597.
6. Connor TJ, Kelly JP, Leonard BE: Forced swim test-induced endocrine and immune changes in the rat: effect of subacute desipramine treatment. *Pharmacol Biochem Behav*, 1998, 59, 171–177.
7. Cryan JF, Page ME, Lucki I: Differential behavioral effect of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl)*, 2005, 182, 335–344.
8. Detke MJ, Lucki I: Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test. The effects of water depth. *Behav Brain Res*, 1995, 73, 43–46.
9. Detke MJ, Rickels M, Lucki I: Active behaviors in the rat forced swimming test differentially produced by seroto-

- nergic and noradrenergic antidepressants. *Psychopharmacology (Berl)*, 1995, 121, 66–72.
10. Dimitriou EC, Dimitriou CE: Bupirone augmentation of antidepressant therapy. *J Clin Psychopharmacol*, 1998, 18, 465–469.
 11. Duncan GE, Knapp DJ, Carson SW, Breese GR: Differential effects of chronic antidepressant treatment on swim stress- and fluoxetine-induced secretion of corticosterone and progesterone. *J Pharmacol Exp Ther*, 1998, 285, 579–589.
 12. Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, Fletcher A: A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY 100635. *Eur J Pharmacol*, 1995, 281, 81–88.
 13. Goodwin GM, DeSouza RJ, Green AR: Attenuation by electroconvulsive shock and antidepressant drugs of the 5-HT_{1A} receptor-mediated hypothermia and serotonin syndrome produced by 8-OH-DPAT in the rat. *Psychopharmacology (Berl)*, 1987, 91, 500–505.
 14. Healy DG, Harkin A, Cryan JF, Kelly JP, Leonard BE: Metyrapone displays antidepressant-like properties in preclinical paradigms. *Psychopharmacology (Berl)*, 1999, 145, 203–208.
 15. Heuser IJE, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Dettling M, Yassouridis A, Holsboer F: Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and normal comparison subjects. *Am J Psychiatry*, 1996, 153, 93–99.
 16. Holsboer F: The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*, 2000, 23, 477–501.
 17. Holsboer F, Barden N: Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocrine Rev*, 1996, 17, 187–205.
 18. Holsboer F, Gerken A, Stalla GK, Muller O: ACTH, cortisol and corticosterone output after ovine corticotropin-releasing factor challenge during depression and after recovery. *Biol Psychiatry*, 1985, 20, 276–286.
 19. Jahn H, Schick M, Kiefer F, Kellner M, Yassouridis A, Wiedemann K: Metyrapone as additive treatment in major depression: a double-blind and placebo-controlled trial. *Arch Gen Psychiatry*, 2004, 61, 1235–1244.
 20. Janssen PAJ, Jageneau AHM, Schellekens KHL: Chemistry and pharmacology of compounds related to 4-(4-hydroxy-4-phenyl-piperidino)-butyrophenone. Part IV. Influence of haloperidol (R 1625) or chlorpromazine on the behaviour of rats in unfamiliar “open field” situation. *Psychopharmacologia*, 1960, 1, 389–392.
 21. Jeffcoate WJ, Silverstone JT, Edwards CR, Besser GM: Psychiatric manifestations of Cushing’s syndrome: response to lowering of plasma cortisol. *Q J Med*, 1979, 48, 465–472.
 22. Karandrea D, Kittas C, Kitraki E: Forced swimming differential affects male and female brain corticosteroid receptors. *Neuroendocrinology*, 2002, 75, 217–226.
 23. Kennett GA, Dickinson SL, Curzon G: Central serotonergic responses and behavioral adaptation to repeated immobilization: the effect of the corticosterone synthesis inhibitor metyrapone. *Eur J Pharmacol*, 1985, 119, 143–152.
 24. Korstein SG, Schneider RK: Clinical features of treatment-resistant depression. *J Clin Psychiatry*, 2001, 62, Suppl 16, 18–25.
 25. Kuroda Y, Watanabe Y, McEwen BS: Tianeptine decreases both serotonin transporter mRNA and binding sites in rat brain. *Eur J Pharmacol*, 1994, 268, R3–R5.
 26. Lesch KP, Aulakh CS, Wolozin BL, Tolliver TJ, Hill JL, Murphy DL: Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Mol Brain Res*, 1993, 17, 31–35.
 27. Linkowski P, Mendlewicz J, LeClerc R, Brasseur M, Hubain P, Goldstein J, Copinschi G, Van Cauter E: The 24-hour profile of ACTH and cortisol in major depressive illness. *J Clin Endocrinol Metab*, 1985, 61, 429–438.
 28. Maes M, Vandoolaeghe E, Desnyder R: Efficacy of treatment with trazodone in combination with pindolol or fluoxetine in major depression. *J Affect Disord*, 1996, 41, 201–210.
 29. Majewska MD: Neurosteroids: endogenous bimodal modulators of the GABA_A receptor. Mechanism of action and physiological significance. *Prog Neurobiol*, 1992, 38, 379–395.
 30. Molodavkin GM, Voronina TA, Meletova OK: Comparative study of the antidepressant and anxiolytic activity of fluoxetine and tianeptine (Russian). *Eksp Klin Farmakol*, 2005, 68, 10–12.
 31. Murphy BEP: Antiglucocorticoid therapies in major depression: a review. *Psychoendocrinology*, 1997, 22, Suppl 1, S125–S132.
 32. Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD et al.: Elevated concentrations of CRF corticotropin releasing factor-like immunoreactivity in depressed patients. *Science*, 1984, 226, 1342–1344.
 33. Nowakowska E, Kuś K, Chodera A, Rybakowski J: Behavioral effects of fluoxetine and tianeptine, two antidepressants with opposite action mechanisms, in rats. *Arzneimittelforschung*, 2000, 50, 5–10.
 34. O’Brien D, Skelton KH, Owens MJ, Nemeroff CB: Are CRF receptor antagonists potential antidepressants? *Hum Psychopharmacol*, 2001, 16, 81–87.
 35. Pariante CM, Miller AH: Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry*, 2001, 49, 391–404.
 36. Porsolt RD, Anton G, Blavet N, Jalfre M: Behavioral despair in rats, a new model sensitive to antidepressant treatment. *Eur J Pharmacol*, 1978, 47, 379–391.
 37. Price LH, Malison RT, McDougle CJ, Pelton GH: Antiglucocorticoids as treatments for depression. Rationale for use and therapeutic potential. *CNS Drugs*, 1996, 5, 311–320.
 38. Raven PW, O’Dwyer A-M, Taylor NF, Checkley SA: The relationship between the effect of metyrapone treatment on depressed mood and urinary steroid profiles. *Psychoneuroendocrinology*, 1996, 21, 277–286.
 39. Rogóż Z: Effect of repeated co-treatment with imipramine and metyrapone on the behavioral reactivity

-
- of the central serotonin, dopamine and α_1 -adrenergic system in rats. *Pharmacol Rep*, 2007, 59, 588–594.
40. RogóŹ Z, Budziszewska B, Kubera M, Basta-Kaim A, Jaworska-Feil L, Skuza G, Lasoń W: Effects of combined treatment with imipramine and metyrapone on the immobility time, the activity of hypothalamo-pituitary-adrenocortical axis and immunological parameters in the forced swimming test in the rat. *J Physiol Pharmacol*, 2005, 56, 49–61.
41. RogóŹ Z, Skuza G: Mechanism of synergistic action following co-treatment with pramipexole and fluoxetine or sertraline in the forced swimming test in rats. *Pharmacol Rep*, 2006, 58, 493–500.
42. RogóŹ Z, Skuza G, Daniel WA, Wójcikowski J, Dudek D, Wróbel A: Amantadine as an additive treatment in patients suffering from drug-resistant unipolar depression. *Pharmacol Rep*, 2007, 59, 778–784.
43. RogóŹ Z, Skuza G, Wójcikowski J, Daniel WA: Effect of combined treatment with imipramine and metyrapone in the forced swimming test in rats. Behavioral and pharmacokinetic studies. *Pol J Pharmacol*, 2003, 55, 993–999.
44. RogóŹ Z, Skuza G, Wójcikowski J, Daniel WA, Wróbel A, Dudek D, Zięba A: Effect of metyrapone supplementation on imipramine therapy in patients with treatment-resistant unipolar depression. *Pol J Pharmacol*, 2004, 56, 849–855.
45. Roozendaal B, Bohus B, McGaugh JL: Dose-dependent suppression of adrenocortical activity with metyrapone: effects on emotion and memory. *Psychoendocrinology*, 1996, 21, 681–693.
46. Satoh T, Yamada K, Tsuboi M: Effect of imipramine on serum corticosterone levels in forced swimming rats. *Res Commun Psychol Psychiatr Behav*, 1985, 10, 235–238.
47. Watanabe Y, Sakai RR, McEwen BS, Mendelson S: Stress and antidepressant effects on hippocampal and cortical 5-HT_{1A} and 5-HT₂ receptors and transport sites for serotonin. *Brain Res*, 1993, 615, 87–94.

Received:

June 30, 2008; in revised form: November 30, 2008.