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Effect of metyrapone on the fluoxetine-induced change in extracellular dopamine, serotonin and their metabolites in the rat frontal cortex

Zofia Rogóż, Krystyna Gołembiowska

Department of Pharmacology, 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 the hyperactivity of the hypothalamic-pituitary-adrenocortical axis, and glucocorticoid synthesis inhibitors have been shown to exert antidepressant action. Metyrapone (an inhibitor of the enzyme 11-β-hydroxylase) has been found to be effective as an adjunctive therapy in combination with other antidepressants (ADs) in both treatment-resistant depression and animal models. To understand the mechanism of the clinical efficacy of a combination of an AD and metyrapone in treatment-resistant depression, the present study was aimed at determining the influence of fluoxetine (FLU; a selective serotonin reuptake inhibitor) and metyrapone, given separately or jointly, on the extracellular level of dopamine (DA), serotonin (5-HT) and their metabolites in rat frontal cortex of freely moving rats using microdialysis and high performance liquid chromatography (HPLC) with electrochemical detection. FLU (10 mg/kg) given alone increased the extracellular level of DA and 5-HT in the rat frontal cortex. Metyrapone (100 mg/kg) alone did not change the level of monoamines. A combination of FLU and metyrapone produced the same change in the efflux of both DA and 5-HT as did FLU alone. However, the latter combination (FLU and metyrapone) produced significantly bigger increases in the levels of extracellullar DA metabolites (3,4-dihydroxyphenylacetic acid, homovanillic acid) and a 5-HT metabolite (5-hydroxyindoleacetic acid) than did FLU alone. The above findings suggest that – among other mechanisms – increases in the levels of extracellullar DA and 5-HT metabolites may play a role in the enhancement of FLU efficacy by metyrapone, and may be of crucial importance to the pharmacotherapy of drug-resistant depression.

Key words:
fluoxetine, metyrapone, monoamines, microdialysis, rats

Introduction

Major depression is frequently associated with the hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis [14, 18, 25, 28–30]. A large number of data indicate that such hyperactivity may be induced by a decreased inhibitory feedback mechanism [11, 33]. In fact, the synthetic glucocorticoid dexamethasone is less potent in lowering cortisol level (basal and that induced by CRH) in the blood of depressed patients than of healthy subjects [11, 12]. The dysfunction of the HPA axis is corrected during a 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 [11, 13]. The currently used antidepressant drugs (ADs) given as a monotherapy show therapeutic efficacy in around 60–70% of depressive

patients [e.g., 24, 48]. Therefore, to improve the therapy, a combination of ADs belonging to different pharmacological groups, or a combination of an AD and a substance that can enhance its effect is used in the clinic [e.g., 2, 7, 26, 49]. Among agents that are expected to potentiate the efficacy of ADs are inhibitors of glucocorticoid synthesis. In fact, they have already been shown to have antidepressant-like properties in some animal models of depression [1, 10]. Also clinical studies have demonstrated some antidepressant effects of metyrapone, aminoglutethimide and ketoconazole; however, the latter drugs are used mainly in drug-resistant depression [28, 38, 39]. To date, in the clinic, glucocorticoid synthesis inhibitors or antagonists of glucocorticoid receptors have been administered alone in relatively high doses, so their side-effects are occasionally observed [38]. A combination of a glucocorticoid synthesis inhibitor and an AD should help to reduce their doses and, in consequence, also their side-effects. Of the glucocorticoid synthesis inhibitors, metyrapone (an inhibitor of the enzyme 11-β-hydroxylase) has the weakest effect on gonadal hormone levels [39]. We found previously that combined treatment with ADs and metyrapone produced a more potent antidepressant-like effect than did either of the drugs given alone to rats in the forced swimming test [40–43]. Additionally, other studies indicated that joint administration of ADs and metyrapone led to clinical improvement [17, 44]. To understand the mechanism of clinical efficacy of an AD and metyrapone combination therapy in treatment-resistant depression, in the present study we assessed the effect of fluoxetine (FLU), a selective serotonin reuptake inhibitor (SSRI), and metyrapone – given separately or jointly – on the extracellular level of dopamine (DA), serotonin (5-HT) and their metabolites in the frontal cortex of freely moving rats using a microdialysis.

Materials and Methods

Animals

Microdialysis studies were conducted on male Wistar rats (270–300 g) (Charles River Laboratories, Germany). The rats were housed in temperature- and humidity-controlled rooms on a 12-hour light/dark cycle (the light on at 7 a.m.) with an *ad libitum* access to filtered tap water and standard pelleted laboratory chow throughout the experiment. The experimental procedures and housing conditions were in strict accordance with the Polish state regulations concerning experiments on animals (DZ. U. 05.33.289). All the experimental protocols were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków, Poland.

Microdialysis study

The rats were anesthetized with ketamine (75 mg/kg, *im*) and xylazine (10 mg/kg, *im*) and placed in stereotaxic apparatus (David Kopf Instruments, CA, USA). Their skulls were exposed and small holes were drilled therein to insert microdialysis probes in the frontal cortex using the following coordinates: 2.9 mm anterior from the bregma, 0.8 mm lateral from the sagittal suture, –4.6 mm ventral from the dural surface. For cortical recording, microdialysis probes were constructed by inserting two fused silica tubes (30 and 35 mm long, 150 μm outside diameter (o.d.) (Polymicro Technologies Inc., Phoenix, AZ, USA) into a microdialysis fibre (220 μm o.d.; AN69, Hospal, Bologna, Italy). The tube assembly was placed in a peak cannula (0.3 mm o.d., 6 mm in long) forming a haft of the probe. Portions of inlet and outlet tubes were individually placed inside a polyethylene PE-10 tubing and glued. The free end of a dialysis fibre was sealed, and 3 mm of its exposed length were used for a dialysis in the frontal cortex. All the probes were connected to a syringe pump (BAS, IN, USA) which delivered an artificial cerebrospinal fluid composed of [mM]: NaCl 145, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2; pH = 7.4 at a flow rate of 2 μl/min. Baseline samples were collected every 30 min after the washout period to obtain a stable extracellular neurotransmitter level. The appropriate drugs (FLU, 10 mg/kg and metyrapone, 100 mg/kg) dissolved in distilled water, were then administered intraperitoneally (*ip*) in a volume of 2 ml/kg separately or jointly, and dialyzate fractions were collected every 30 min for 4 h. At the end of the experiment, the rats were sacrificed and their brains were histologically examined to validate the probe placement.