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Short communication

Effects of PB190 and PB212, new σ receptor ligands, on glucocorticoid receptor-mediated gene transcription in LMCAT cells

Grażyna Skuza[1], Magdalena Szymańska[2], Bogusława Budziszewska[2,3], Carmen Abate[4], Francesco Berardi[4]

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

[3]Department of Biochemical Toxicology, Chair of Toxicology, Jagiellonian University, Medical College, Medyczna 9, PL 30-688 Kraków, Poland

[4]Dipartimento Farmacochimico, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy

Correspondence: Grażyna Skuza, e-mail: skuza@if-pan.krakow.pl

Abstract:
The hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis is often observed in patients with major depression. It has even been implicated in the pathophysiology of this disease. Some antidepressant drugs (ADs) inhibit glucocorticoid receptor (GR) function under *in vitro* conditions. The σ_1 receptor agonists reveal potential antidepressant activity in animals, moreover, igmesine is promising as an AD in humans. As already shown, σ receptors are involved in stress-induced responses (e.g., conditioned fear stress in mice). The aim of the present study was to find out whether the new selective σ receptor ligands, PB190 and PB212, are able to affect directly the endocrine system activity. To this end, we evaluated their influence on GR function in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). Fluvoxamine, a selective serotonin reuptake inhibitor, recognized as a σ_1 receptor agonist was used for comparison. The obtained results showed that both PB190 and PB212 (potential σ_1 receptor agonist and antagonist, respectively) like fluvoxamine, decreased the corticosterone-induced CAT activity in a concentration-dependent manner. The significance of this fact remains ambiguous and requires further studies.

Key words:
selective σ ligands, PB190, PB212, glucocorticoid-mediated gene transcription, fibroblast cells

Introduction

It has been demonstrated that several antidepressant drugs (ADs) have the affinity for σ receptors in nanomolar range and that this fact may be relevant to their mechanism of antidepressant action [for review: 4, 8, 14, 24]. The σ receptor ligands (especially σ_1 receptor agonists, e.g., igmesine, SA4503, (+)-pentazocine, UMB23 and UMB82) reveal potential antidepressant activity in animal models of depression, e.g., the forced swim test, tail suspension test, conditioned fear stress [17, 22, 29, 31]. Preclinical studies have shown that targeting σ receptors alone is sufficient (but not requisite) for production of antidepressant-like actions. More-

over, the high-affinity σ receptor agonist igmesine is promising as an AD in humans (phase II clinical trials) [30]. It is believed that σ receptors represent an initial target (similarly to monoamine transporters) in a cascade of events that results finally in an antidepressant action. However, this mechanism of action of σ receptor ligands is not clear in detail [e.g., 8, 18].

In patients suffering from major depression, a hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) axis is often observed. It has even been implicated in the pathophysiology of this disease [1, 15, 19]. Recent studies have indicated that ADs inhibit glucocorticoid receptor (GR) function under *in vitro* conditions [5, 6, 21].

Hitherto existing data indicate that hyperactivity of HPA axis in major depression can be induced by impairment of the feedback inhibition mechanism [10, 20]. In fact, the synthetic glucocorticoid, dexamethasone, is less potent in lowering blood cortisol level (basal and CRH-induced) in depressed patients than in healthy subjects [9, 12]. The dysfunction of the HPA axis is corrected during a clinically effective therapy with ADs [9, 11, 22]. On the other hand, some recent clinical studies have revealed that the inhibitors of cortisol synthesis (metyrapone, ketoconazole, aminoglutethimide) and CRH_{1R} receptor antagonist show antidepressant effect [10].

As previously shown, some ADs inhibit the HPA axis activity in two different, independent ways: they increase the GR level in the CNS and/or enhance the GR-mediated feedback inhibition. In consequence, ADs are able not only to decrease the cortisol/corticosterone level but also to inhibit corticosterone receptor-mediated transcription of some genes. Moreover, some behavioral changes (e.g., impairments in food consumption, sleep, learning and memory) have been found in HPA-hyperactive transgenic mice (with reduced GR expression mainly in neuronal tissue) [5].

Our previous results indicated that the selective σ_1 receptor agonists, SA 4503, PRE 084 and di-*o*-tolylguanidine (DTG), $\sigma_{1/2}$ receptor agonist, which predominantly showed antidepressant-like activity in behavioral models, were without effect in an *in vitro* model. On the contrary, BD 1047, SM 21 and rimcazole, σ receptor antagonists, in a statistically significant manner inhibited the corticosterone-induced gene transcription [27].

The aim of the present study was to find out whether the selective σ_1 receptor ligands, PB190 and PB212, potential σ_1 receptor agonist and antagonist respectively – are able to affect directly the endocrine system activity [2]. To this end, we evaluated their influence on GR function in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). Fluvoxamine, an AD representing the selective serotonin reuptake inhibitors (SSRIs), with σ_1-preferring receptor affinity, was used for comparison.

Materials and Methods

Cell culture

Effects of drugs on GR-mediated gene expression were determined in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) reporter plasmid (LMCAT cells). The LMCAT cell line was generously provided by Dr. E.R. Sanchez (Department of Pharmacology, Medical College of Ohio, Toledo, OH, USA).

Drug treatments

The LMCAT cells (final confluency 80%) were treated for five days with vehicle, PB190, PB212, or fluvoxamine maleate (Tocris Cookson Ltd., UK), used as a reference compound. The drugs were dissolved in water. The control cultures were supplemented with the same amount of an appropriate vehicle. All drugs were added at final concentrations of 1, 3, 10 and 30 μM. The medium and drugs were changed once over the course of a 5-day culture. Gene transcription was stimulated by adding 1 μM corticosterone for 2 h before harvesting cells.

Chloramphenicol acetyltransferase (CAT) activity assay

CAT activity was determined as described previously [6]. Cell lysates were prepared by a freezing/thawing procedure. To determine CAT activity, aliquots of lysate (after heating at 60°C for 10 min) were incubated in 0.25 M Tris-HCl buffer (pH = 7.8) with 0.25 μCi D-threo-[dichloroacetyl-1-^{14}C]-chloramphenicol and 0.2 mM *n*-butyryl coenzyme A at 37°C for 1 h.

The butyrylated forms of chloramphenicol (in direct proportion to the CAT gene expression) were extracted twice with xylene (mixture of isomers), washed with 0.25 M Tris-HCl buffer, and radioactivity was measured in a β-counter (Beckmann LS 335 liquid scintillation counter). The results are presented as dpm of a butyrylated fraction of chloramphenicol per 10 μg of protein per 1 h of incubation. The protein concentration in cell lysates was determined by the method of Lowry et al. [16].

Statistical analysis

The data are presented as the mean ± SEM of four to five independent experiments (in duplicate wells), and the significance of differences between the means was evaluated by the Duncan's test following one-way analysis of variance.

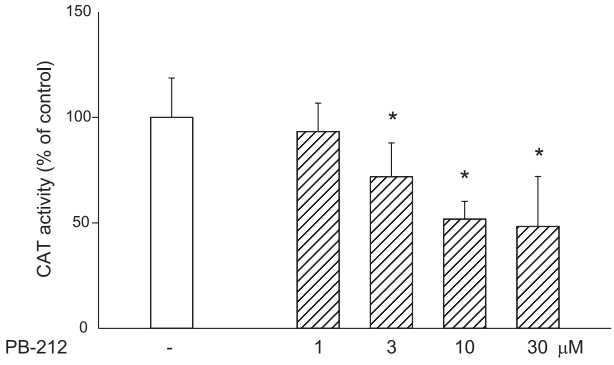

Fig. 2. The effect of PB212 on corticosterone (1 μM, 2 h)-induced CAT activity in LMCAT cells. PB212 was used at concentrations of 1, 3, 10 and 30 μM for 5 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for assays of CAT enzyme activity. The data are presented as the means ± SEM (% of control). The significance of differences between the means was evaluated by Duncan's test following a one-way analysis of variance (* $p < 0.05$ *vs.* respective control)

Results and Discussion

Addition of corticosterone at a concentration of 1 μM for 2 h increased CAT activity about 35-fold. As we described previously, the effect of corticosterone was completely blocked by addition of 10 μM RU 38486, a specific antagonist of the type II GR, which confirms involvement of the glucocorticoid receptor in this response [6].

None of the drugs under study affected the low, non-stimulated CAT activity (data not shown). Treatment of cells with PB190 at concentrations 1–30 μM concentration-dependently decreased the corticosterone-induced CAT activity (Fig. 1). PB212 decreased CAT activity at concentrations of 3, 10 and 30 μM, while the lowest concentration (1 μM) was ineffective (Fig. 2). Fluvoxamine, used for comparison, at concentrations of 3, 10 and 30 μM statistically significantly inhibited corticosterone-induced gene transcription (its lowest concentration was inactive) (Fig. 3).

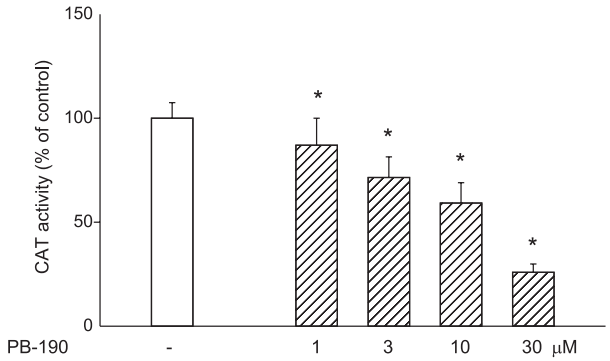

Fig. 1. The effect of PB190 on corticosterone (1 μM, 2 h)-induced CAT activity in LMCAT cells. PB190 was used at concentrations of 1, 3, 10 and 30 μM for 5 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for assays of CAT enzyme activity. The data are presented as the means ± SEM (% of control). The significance of differences between the means was evaluated by Duncan's test following a one-way analysis of variance (* $p < 0.05$ *vs.* respective control)

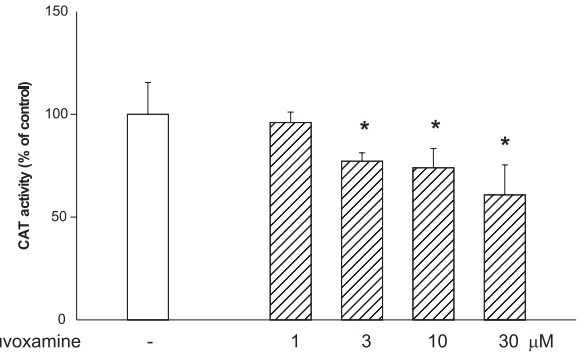

Fig. 3. The effect of fluvoxamine on corticosterone (1 μM)-induced CAT activity in LMCAT cells. Fluvoxamine was used at concentrations of 1, 3, 10 and 30 μM for 5 days. Corticosterone (1 μM) was added 2 h before harvesting the cells for assays of CAT enzyme activity. The data are presented as the mean ± SEM (% of control). The significance of differences between the means was evaluated by Duncan's test following a one-way analysis of variance (* $p < 0.05$ *vs.* respective control)

Among all the piperidines synthesized by Berardi group [2, 3, 7], PB212 and its corresponding tetralin counterpart PB190 (naphthalene and its tetralin counterpart) were approved as the compounds with an optimal binding profile, displaying a good affinity and selectivity for σ_1 receptors. The K_i values of PB212 for σ_1 and σ_2 receptors are 0.030 ± 0.013 and 17.9 ± 5.3 (nM), respectively, for PB190 0.42 ± 0.04 and 36.3 ± 5.2, respectively. Moreover, they revealed weak or no affinity for many known receptors [2, 3, 7].

Functional assays in rat C6 glioma cells showed an unexpected tendency, *viz.* the tetralin derivatives displayed agonist (pentazocine-like) activity while naphthalene derivatives presented antagonist activity (NE100-like) in the σ_1-mediated antiproliferative assays. The preliminary *in vivo* studies seem to confirm these tendency: PB190 behaves as a σ_1-receptor agonist while PB212 antagonized its activity (Skuza, in preparation).

Although the mechanisms underlying the ability of σ receptor ligands (agonists) to produce antidepressant-like actions have yet to be fully elucidated, many of data suggest that σ receptor agonists stimulate a variety of neural adaptations in the central nervous system that are relevant to antidepressant action. Recently, it has been shown that σ_1 receptors up-regulate the release of brain derived neurotrophic factor (BDNF) which has been hypothesized to contribute to the action of ADs [13, 32]. Moreover, fluvoxamine and SA 4503, σ_1 receptor agonists, potentiate nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells [28]. The potentiation by fluvoxamine and other receptor agonists (e.g., SA 4503) was blocked by NE-100, the σ_1 receptor antagonist.

It was shown that ADs (e.g., imipramine, desipramine, amitriptyline, fluoxetine, mianserin and tianeptine) inhibited corticosterone-induced gene transcription in a concentration- and time-dependent manner [5]. Moreover, imipramine decreases binding of corticosterone-receptor complex to DNA and its inhibitory effect depends partly on the PLC/PKC pathway [6]. Our previous results indicated that σ_1 receptor agonists, SA 4503 and PRE 084, failed to affect corticosterone-induced gene transcription. DTG, σ_1/σ_2 receptor agonist, acted alike, but its lowest concentration slightly increased the effect of corticosterone on GR-mediated gene transcription. Nevertheless, all the studied compounds exerted antidepressant-like activity in behavioral models (e.g., forced swim test or tail suspension test in rats and mice) [17, 25, 26]. The obtained results showed that, in contrast to σ_1 receptor agonists (SA 4503, PRE-084, DTG) studied previously [27], claimed σ_1 agonist PB190 and claimed σ_1 antagonist PB212, behave like fluvoxamine, decreasing the corticosterone-induced CAT activity in a concentration-dependent manner. The significance of this fact remains ambiguous and requires further studies.

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