European Journal of Pharmacology 608 (2009) 7–13

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and Cellular Pharmacology

Gender-related differences in the effects of antidepressant imipramine on glucocorticoid receptor binding properties and association with heat shock proteins in the rat liver and kidney

Ivana Elaković, Jelena Brkljačić, Gordana Matić *

University of Belgrade, Institute for Biological Research "Siniša Stanković", Department of Biochemistry, Belgrade, Serbia

ARTICLE INFO

Article history:
Received 24 October 2008
Received in revised form 28 January 2009
Accepted 9 February 2009
Available online 5 March 2009

Keywords:
Glucocorticoid receptor
Antidepressant
Imipramine
Gender difference
Liver
Kidney

ABSTRACT

Gender-related differences in susceptibility to stress and stress-related disorders such as depression, and in response to treatment with antidepressants have been observed, but the underlying molecular mechanisms are still unknown. Considering the role of glucocorticoid hormones in the systemic reaction against stress and in pathogenesis of depression, the aim of the present work was to study gender-related differences in glucocorticoid signaling and in response of this system to a typical antidepressant drug, imipramine. Gender-related differences in glucocorticoid receptor functional properties were assessed using hepatic and renal whole cell extracts of female and male rats before and after long-term imipramine treatment. The receptor's hormone-binding parameters, B_{max} and K_D, were determined by radioligand binding assay, the glucocorticoid receptor and heat shock proteins (Hsp70 and Hsp90) levels by quantitative immunoblotting, and the interaction of these proteins within glucocorticoid receptor heterocomplex by co-immunoprecipitation. Glucocorticoid receptor binding potency (B_{max}/K_D ratio) was significantly higher in males than females both before and after treatment with imipramine. Gender-specific changes in the glucocorticoid receptor binding parameters in the examined tissues were observed in response to imipramine, and were found to be associated with alterations in the receptor interaction with Hsp70 and Hsp90. The results of the study point to sexual dimorphism in the glucocorticoid signaling and imply that glucocorticoid receptor functional alterations contribute to gender-related differences in vulnerability to stress and stress-related disorders, and in response to antidepressant drugs.

1. Introduction

Antidepressant drugs, acting on different monoamine systems, are broadly used in the treatment of major depressive disorder, but no correlation between the therapeutic efficacy of a drug and its specific monoamine target was found. Moreover, these drugs affect synaptic concentration of monoamines rapidly, while the first signs of clinical improvement are observed only 2–3 weeks after the onset of the treatment. These findings suggest that molecular systems other than monoaminergic might also be implicated in the mechanism of action of these drugs and in pathogenesis of depression. In numerous studies, the effects of antidepressant drugs on glucocorticoid receptor are proposed to be involved in the clinical resolution of depression, but the underlying molecular mechanisms are still unknown.

A majority of depressed patients exhibit hyperactivity of the hypothalamo-pituitary-adrenocortical (HPA) axis, which is considered to be a consequence of hypersecretion of corticotropin-releasing hormone (CRH) from the hypothalamus (Holsboer, 2000). One of the suggested mechanisms underlying these alterations is an impaired feedback inhibition of CRH release by endogenous glucocorticoids (McEwen et al., 1986). This is supported by numerous clinical studies showing attenuated suppression of cortisol secretion following the administration of dexamethasone, as well as a lack of inhibition of adrenocorticotropic hormone responses to CRH after dexamethasone pretreatment (Holsboer, 2000; Pariante and Miller, 2001). Consistent with these findings are the observations that successful antidepressant treatment normalizes HPA axis function in depressed patients, which is reflected in normalization of CRH and cortisol levels and the response to dexamethasone challenge. On the contrary, the persistent non-suppressive state of the HPA axis in clinically remitted patients has been associated with the higher rate of depression relapse (Ribeiro et al., 1993).

Both chronic and acute stress are factors commonly recognized in the etiology of depression (Van Praag, 2004), and the incidence of depression and other stress-related affective disorders is significantly greater in women than in men (Kuehner, 2003). It is reported that female and male patients, suffering from major depression, exert different extent of cortisol suppression after dexamethasone administration (Osuch et al., 2001), and that estrogen stimulates CRH synthesis in the hypothalamus of rats, while decreasing glucocorticoid

* Corresponding author. Department of Biochemistry, Institute for Biological Research "Siniša Stanković", 142 Despot Stefan Blvd., 11060 Belgrade, Serbia. Tel.: +381 11 2078303, +381 63 8121713; fax: +381 11 2761433.
E-mail address: gormatic@ibiss.bg.ac.yu (G. Matić).

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2009.02.038

receptor mRNA levels in the hippocampus (Vamvakopoulos and Chrousos, 1993). Moreover, estrogen has been demonstrated to impair glucocorticoid negative feedback (Burgess and Handa, 1992), whereas testosterone inhibits HPA axis activity (Seale et al., 2004). Acting this way, estrogen might facilitate the onset and progression of stress-induced affective disorders. Apart from gender-related differences in the prevalence of depression, the differences in treatment response to antidepressants were also observed. While tricyclic antidepressant drugs are more efficient in men, women respond better to selective serotonin reuptake inhibitors (Kornstein et al., 2000). Tricyclic antidepressants are metabolized in the liver by two main routes: N-demethylation and ring hydroxylation, and metabolites retain biological activity. Inactivation of these drugs by glucuronide conjugation mainly occurs in the liver, though some reactions take place also in the kidney. Gender-related differences in the hepatic antidepressant drug metabolism have been reported, suggesting different impact of gonadal steroids on expression of microsomal enzymes involved in metabolism of xenobiotics (Dannan et al., 1986).

There are many experimental data pointing out that antidepressant drugs can exert their effects through modulation of glucocorticoid receptor. It is well documented that different antidepressants modulate glucocorticoid receptor expression both at mRNA and protein level in the brain (Pepin et al., 1989; Pariante and Miller, 2001). Findings showing the ability of antidepressants to increase glucocorticoid receptor mRNA level in rats after complete neurotoxic lesion of norepinephrine neurons (Rossby et al., 1995), in cell cultures which do not contain monoaminergic neurons (Pepin et al., 1989) and especially in fibroblasts, cells that do not secret catecholamine (Pepin et al., 1992), corroborate the idea that pharmacologically unrelated antidepressants may directly affect glucocorticoid receptor, without influencing serotonin and noradrenalin reuptake. Apart from increasing concentration, antidepressants have also been found to affect glucocorticoid receptor-mediated gene transcription (Pepin et al., 1992; Pariante et al., 1997; Budziszewska et al., 2000; Okuyama-Tamura et al., 2003; Otczyk et al., 2008). Pariante et al. (2001), however, demonstrated that various antidepressants inhibit steroid transporters on the blood brain barrier, which pump glucocorticoids out of the brain and thus, increase availability of these hormones to bind to their receptors. In this light, peripheral tissues, in which the effects of antidepressants on monoaminergic transmission and their impact on the blood brain barrier steroid transporters are excluded, might serve as a useful model system for investigating direct effects of these drugs on glucocorticoid receptor function.

Glucocorticoid receptor is a hormone-activated transcription factor. In the absence of hormone it is predominantly located in the cytoplasm of target cells within large multiprotein chaperone complexes, the key components of which are heat shock proteins (Hsps) Hsp90 and Hsp70 (reviewed in Pratt and Toft, 1997; Grad and Picard, 2007). In association with the glucocorticoid receptor, Hsp90 serves to facilitate the receptor's folding, to maintain the hormone-binding domain in a high-affinity steroid-binding conformation, to stabilize the receptor against proteolytic degradation, as well as to enable its transactivation activity and intracellular shuttling. As for Hsp70, it is required for glucocorticoid receptor heterocomplex assembly and maturation of the receptor's hormone-binding ability.

Glucocorticoid receptor functional activity can be regulated at several distinct steps, including: synthesis and degradation of the receptor protein, availability of the hormone, the receptor's hormone-binding capacity and affinity, association/dissociation of the receptor with chaperon and co-chaperon proteins including Hsps, translocation of the hormone–receptor complexes to the nucleus, the receptor binding to DNA and its transactivation activity. Each of these steps is a possible target for antidepressant drug action, but the experimental data on the effects of antidepressants on these processes are rather scarce.

The aim of the present work was to examine gender-related differences in the effects of a typical tricyclic antidepressant drug, imipramine, on glucocorticoid receptor hormone-binding properties. Gender-specific influence of a long-term imipramine administration on the glucocorticoid receptor hormone-binding capacity and affinity in the rat liver and kidney was observed. The imipramine-induced changes in the receptor hormone-binding activity were associated with the alterations in the receptor assembly with Hsp70 and Hsp90 in hepatic whole cell extracts.

2. Materials and methods

2.1. Materials

Imipramine hydrochloride, unlabelled dexamethasone, Protein A-Sepharose and mouse anti-β-actin monoclonal antibody (AC-15) were purchased from Sigma Chemicals (St. Louis, MO, USA). [^{3}H]Dexamethasone (specific activity 35.0 Ci/mmol), PVDF membrane and ECF Western blotting reagent pack, containing anti-mouse (IgG + IgM) and anti-rabbit IgG alkaline phosphatase-linked whole antibodies, and ECF substrate were obtained from Amersham Pharmacia Biotech, UK. Anti-glucocorticoid receptor antibodies, rabbit polyclonal (PA1-511A), and mouse monoclonal (BuGR2), were the products of Affinity BioReagents (Golden, CO, USA), while anti-Hsp70/Hsc70 (N27F3-4) and anti-Hsp90 (AC88) monoclonal antibodies were obtained from StressGen (Canada).

2.2. Animals and treatment

Male and female Wistar rats, bred in our laboratory, were 2.5 months old at the beginning of the treatment. Animals were housed four per cage, at 22 °C, with a 12 h light/dark cycle (lights on at 07:00 h). Food and drinking water were available *ad libitum*. Imipramine hydrochloride dissolved in 0.9% saline was administered intraperitoneally at a daily dose of 10 mg/kg b.w. at 09:00 h for 21 days. Both female and male control groups received 0.9% saline under the same conditions as matching experimental groups. Vaginal smears were microscopically analyzed for determination of the estrous cycle stage, and all females selected for the experiments were in the diestrous phase on the day of sacrifice. Animals were weighted weekly. The procedures were complied with the Declaration of Helsinki and were approved by the ethics committee of the Serbian Association for the Use of Animals in Research and Education. All the experiments were replicated three times, each time with the new groups of imipramine-treated and untreated females and males. Each group consisted of four animals.

2.3. Tissue collection and preparation of whole cell extracts

Animals were sacrificed by rapid decapitation 24 h after the last imipramine injection. Livers were perfused with cold 0.9% NaCl before isolation. Tissues from four animals belonging to the same experimental group were pooled and homogenized in 2 vol (w/v) of 15 mM Tris buffer, pH 7.9 at 4 °C, containing 0.25 M sucrose, 16 mM KCl, 15 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 20 mM Na-molybdate, 1 mM dithiothreitol and 0.1 mM phenylmethylsulfonyl-fluoride. The homogenates were treated with ultrasound 3 × 15 s on ice at 1 A and 50/60 Hz, with 30% amplitude (Hielscher Ultrasound Processor) and centrifuged for 60 min at 105,000 g, 4 °C. The floating lipid layers were aspirated and supernatants (whole cell extracts) were stored in liquid nitrogen until use.

2.4. Steroid-binding analysis

Examination of imipramine effects on glucocorticoid receptor equilibrium binding parameters was done by a radioligand–receptor binding assay. Aliquots of whole cell extracts (50 µl) were incubated 18 h at 0 °C with [^{3}H]dexamethasone at seven different concentrations