Neuroscience Letters 533 (2013) 86–89

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Induction of methionine sulfoxide reductase activity by pergolide, pergolide sulfoxide, and *S*-adenosyl-methionine in neuronal cells

Jade M. Franklin, Gonzalo A. Carrasco, Jackob Moskovitz*

Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA

HIGHLIGHTS

- Methionine sulfoxide reductase (Msr) is upregulated in neurons by small compounds.
- Msr activity is upregulated upon treatment with pergolide and pergolide sulfoxide.
- Msr activity is upregulated upon treatment with *S*-adenosyl-methionine.
- The compound-mediated upregulated Msr activity may alleviate neuronal malfunction.

ARTICLE INFO

Article history:
Received 12 September 2012
Received in revised form 25 October 2012
Accepted 12 November 2012

Keywords:
Pergolide
S-adenosyl methionine
Oxidative stress
Post-translational modification
Signal transduction
Antioxidants

ABSTRACT

The reduction of methionine sulfoxide in proteins is facilitated by the methionine sulfoxide reductase (Msr) system. The Msr reduction activity is important for protecting cells from oxidative stress related damages. Indeed, we have recently shown that treatment of cells with *N*-acetyl-methionine sulfoxide can increase Msr activity and protect neuronal cells from amyloid beta toxicity. Thus, in search of other similar Msr-inducing molecules, we examined the effects of pergolide, pergolide sulfoxide, and *S*-adenosyl-methionine on Msr activity in neuronal cells. Treatment of neuronal cells with a physiological range of pergolide and pergolide sulfoxide (0.5–1.0 μM) caused an increase of about 40% in total Msr activity compared with non-treated control cells. This increase in activity correlated with similar increases in methionine sulfoxide reductase A protein expression levels. Similarly, treatment of cells with *S*-adenosyl methionine also increased cellular Msr activity, which was milder compared to increases induced by pergolide and pergolide sulfoxide. We found that all the examined compounds are able to increase cellular Msr activity to levels comparable to *N*-acetyl-methionine sulfoxide treatment. Pergolide, pergolide sulfoxide, and *S*-adenosyl methionine can cross the blood–brain barrier. Therefore, we hypothesize that they can be useful in the treatment of symptoms/pathologies that are associated with reduced Msr activity.

1. Introduction

The methionine sulfoxide reductase (Msr) system is comprised of MsrA and MsrB enzymes that reduce methionine sulfoxide (MetO) to its *S*-form or *R*-form, respectively [15]. Lack of MsrA causes cells and animals to be more vulnerable to oxidative stress and have a shorten life span/survival rate. In contrast, overexpression of recombinant Msrs protects cells from oxidative stress insults and beta-amyloid toxicity [6,7,9]. Recently we were able to induce an increase of total intrinsic Msr activity by either methionine-oxidized beta amyloid peptides or *N*-acetylated methionine sulfoxide compound [11]. These observations prompted us to search for small molecules that will either contain methyl sulfide/sulfoxide group or will be a derivative structure of methionine. The drug pergolide functions as a dopamine receptor agonist for D_2 and D_1 receptors and as a ligand to serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors [4,8]. Pergolide was used to treat Parkinson disease in the United States but this has ceased due to studies linking pergolide with increased rates of valvular dysfunction [1,17,20,22]. However, pergolide is still used in other countries in the treatment of Parkinson's disease, restless leg syndrome, and hyperprolactinemia.

Pergolide sulfoxide is the oxidative derivative of pergolide. Pergolide sulfoxide is used in the current studies because it contains methyl sulfoxide adduct that is also present in the Msr activity inducer, *N*-acetyl-methionine sulfoxide [11]. Noteworthy, the oxidized form of pergolide, pergolide sulfoxide, still maintains its normal functions as neuronal receptor ligand. Furthermore, an additional beneficiary feature of pergolide/perfolide sulfoxide is that it can cross the blood–brain barrier which is critical for

Abbreviations: SAM, *S*-adenosyl-methionine; Msr, methionine sulfoxide reductase; MetO, methionine sulfoxide.

* Corresponding author. Tel.: +1 785 864 3536; fax: +1 785 864 5219.
E-mail address: moskovij@ku.edu (J. Moskovitz).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.11.017

the therapeutic delivery of the compound to the brain. We also investigated the ability of *S*-adenosyl methionine (SAM) to induce Msr activity mainly because it is a derivative of methionine that may upregulate Msr expression and can cross the blood brain barrier. SAM was reported to protect the brain from oxidative stress and Alzheimer's disease symptoms through facilitation of glutathione usage and maintenance of acetylcholine levels, respectively [2,3,21]. It is hypothesized that SAM may upregulate Msr activity as part of a signal transduction pathway, leading to an increased antioxidant capability that protects the brain from oxidative stress insults. To determine the effect of these compounds on Msr activity, we used a neuronal cell model, CLU213 cells that were selected because they have moderate Msr levels of activity, which allows its monitoring. This neuronal cell model endogenously expresses dopamine, serotonin, and cannabinoid receptors (providing various neuronal capabilities of signal transduction pathways).

2. Materials and methods

2.1. Cell culture protocol

A neuronal cell line that expresses dopamine, serotonin and cannabinoid receptors, CLU213 cells, was purchased from Cedarlane Laboratories (Burlington, NC, USA). Pergolide mesylate salt and S-adenosyl methionine were purchased from Sigma (St. Louis, MO). Pergolide sulfoxide was purchased from Carbosynth (Dan Diego, CA).

2.2. Effect of pergolide, pergolide sulfoxide, and S-adenosyl methionine on Msr activity in cell culture

CLU213 cells were grown on 100-mm^2 plates treated with polystyrene (Corning Incorporated, Corning, NY) and maintained in 5% CO_2 at 37 °C, in Dulbecco's modified eagle medium (DMEM; Mediatech Inc, Manassas, VA) containing 10% fetal bovine serum (FBS; Thermo Scientific, Logan, UT). When the cells reached 80% confluence they were washed three times with 1× PBS and incubated in charcoal treated medium prior to treatment. One set of cells was then incubated with either vehicle (ethanol 0.01% final concentration), pergolide (0.1, 0.5, 1, or 10 μM), or pergolide sulfoxide (0.1, 0.5, 1, or 10 μM) for 24 h. Similarly, another set of cells was incubated with either vehicle (sterile double distilled water), or *S*-adenosyl-methionine (0.1, 0.5, 1, or 10 μM), for 24 h. The next day cells were washed three times with 1× PBS and collected for analysis of Msr activity. All treatments were performed in triplicate.

2.3. Methionine sulfoxide reductase (Msr) activity

Post treatment harvested cells were disrupted in PBS in the presence of protease inhibitors cocktail (Roche) at 4 °C. The resulting extracts were spun down (10,0000 × *g*) and the soluble material was collected and analyzed for protein concentration using the Bio-Rad assay. Equal amounts of protein extracts (200 μg) were analyzed for total Msr activity as previously described [12]. Briefly, a reaction mixture (100 μl) containing 25 mM Tris 7.5, 20 mM DTT, 200 μM of dabsyl-MetO, and 200 μg of extracted protein was incubated for one hour at 37 °C. Following the incubation, the reaction was stopped by adding equal volume of acetonitrile. Insoluble material was spun down and the soluble material was subjected to HPLC-based analysis for the presence of dabsyl-Met, using reverse phase C-18 column [12]. The detected peak of dabsyl-Met was integrated and the Msr specific activity was calculated as pmols dabsyl-Met formed per mg protein.

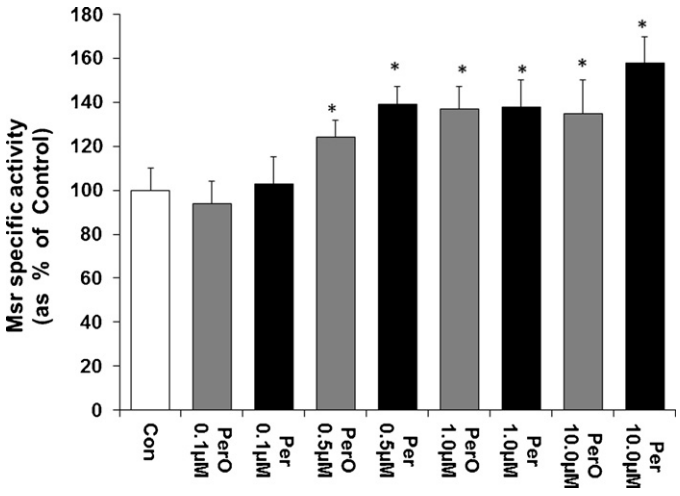

Fig. 1. Pergolide and pergolide sulfoxide effect on Msr activity in neuronal cells. CLU213 cells were grown in 5% CO_2 at 37 °C, in Dulbecco's modified eagle medium containing 10% fetal bovine serum. When the cells reached 80% confluence they were washed three times with PBS and incubated in charcoal treated medium prior to treatment. Cells were then incubated with either vehicle (ethanol 0.01% final concentration), pergolide (0.1, 0.5, 1, or 10 μM), or pergolide Sulfoxide (0.1, 0.5, 1, or 10 μM) for 24 h. Post treatment harvested cells were disrupted in PBS in the presence of protease inhibitors cocktail (Roche) and the resulting extracts were spun down and the soluble material was collected. Equal amounts of protein extracts were analyzed for total Msr activity, using dabsyl-MetO as substrate, as previously described [12] and as described in Materials and Methods. Following an HPLC-based analysis for the presence of dabsyl-Met (see Section 2), the detected peak of dabsyl-Met was integrated and the Msr specific activity was calculated as pmols dabsyl-Met formed per mg protein (the value 200 pmols dabsyl-Met formed per mg protein was defined as 100% specific Msr activity). Abbreviations: Con, control cells treated with vehicle only; Per, pergolide; PerO, pergolide sulfoxide. The experiments were repeated three times. Error bars: Standard deviations. The symbol: * indicates a statistical significance of $P < 0.01$ between Per/PerO and vehicle-treated cells (one-way analysis of variance (ANOVA)).

2.4. Msr expression (MsrA and MsrB)

The expression levels of MsrA and MsrB were monitored by analyzing equal amounts of cell-protein extracts in western blot analyses using primary rabbit anti-MsrA antibodies [10] or rabbit anti-MsrB1 [13] antibodies. The secondary antibodies were HRP-conjugated goat anti rabbit IgG (Bio-Rad, Hercules, CA). Protein loading control was determined by β-actin levels, using primary mouse anti-β-actin antibodies (Abcam, Cambridge, MA). The secondary antibodies for the β-actin antibodies were HRP-conjugated goat anti-mouse IgG antibodies (Bio-Rad, Hercules, CA). Following exposure of the western blots to X-ray film, the density of resulting detected Msr's and β-actin protein bands were determined by Image-J program (NIH, Bethesda, MD).

3. Results and discussion

In our first studies we measured the effect of three small molecules that can cross the blood brain barrier on Msr activity and expression in neuronal cell culture. Both pergolide and pergolide sulfoxide significantly upregulated total Msr activity at physiological concentrations of 0.5 μM to 1 μM, as well as at a higher concentration of 10 μM ($p < 0.01$; Fig. 1). While pergolide increased Msr activity to about 140% at concentrations of 0.5 μM and 1 μM, pergolide sulfoxide showed a similar effect only starting at concentration of 1.0 μM (Fig. 1). In addition, only pergolide's effect became stronger at 10.0 μM, showing a 158% upregulation of Msr activity compared with vehicle-treated cells only (control) (Fig. 1). To determine if the observed increase of Msr activity is associated with increased protein expression of Msr enzymes we measured the expression of MsrA protein by western blot analysis after treatment