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The Glutathione System and its Regulation by Neurohormone Melatonin in the Central Nervous System

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Abstract: The glutathione system includes reduced (GSH) and oxidized (GSSG) forms of glutathione; the enzymes required for its synthesis and recycling, such as gamma-glutamate cysteine ligase (γ-GCL), glutathione synthetase (GS), glutathione reductase (GSR) and gamma glutamyl transpeptidase (γ-GGT); and the enzymes required for its use in metabolism and in mechanisms of defense against free radical-induced oxidative damage, such as glutathione s-transferases (GSTs) and glutathione peroxidases (GPxs). Glutathione functions in the central nervous system (CNS) include maintenance of neurotransmitters, membrane protection, detoxification, metabolic regulation, and modulation of signal transduction. A common pathological hallmark in various neurodegenerative disorders, such as amyotrophic lateral sclerosis and Alzheimer's and Parkinson's diseases, is the increase in oxidative stress and the failure of antioxidant systems, such as the decrease in the GSH content. The administration of exogenous neurohormone melatonin at pharmacological doses has been shown not only to be an effective scavenger of reactive oxygen and nitrogen species but also to enhance the levels of GSH and the expression and activities of the GSH-related enzymes including γ-GCL, GPxs, and GSR. The exact mechanisms by which melatonin regulates the glutathione system are not fully understood. The main purpose of this short review is to discuss evidence relating to the potential common modulation signals between the glutathione system and melatonin in the CNS. The potential regulatory mechanisms and interactions between neurons and non-neuronal cells are also discussed.

Keywords: Glutathione, γ-glutamate cysteine ligase, glutathione peroxidases, melatonin, brain.

INTRODUCTION

Oxidative stress occurs when the production of reactive oxidative species (ROS) exceeds the capacity of cellular antioxidant defenses to remove these toxic species. The central nervous system (CNS) in particular is highly susceptible to damage by ROS for several reasons. First, the CNS has a high rate of oxygen consumption; the brain constitutes about 2% of total body weight but accounts for 20% of total body oxygen usage. Second, the CNS has high iron content, which could be a source (through Fenton and Haber-Weiss reactions) of ROS. Moreover, iron is differentially distributed in some brain areas, and its presence has been shown to increase with the age [1] while the antioxidant defenses decline [2, 3]. Third, the CNS might be especially vulnerable to ROS because it is rich in lipids with unsaturated fatty acids, which are a source for lipid peroxidation (LPO). Finally, the brain has only moderate antioxidant capacity; this limited capacity confers susceptibility to oxidative destruction and might contribute to the development of neurodegenerative disorders [2, 4, 5].

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The role and potential clinical applications of endogenous and exogenous antioxidants for protecting the brain from ROS have been extensively tested but are still debated. Nutritional supplement studies have tested the protection conferred by vitamins E and C or by micronutrients such as selenium either alone or in combination. These studies have shown benefits of nutritional supplements in experimental models, but in clinical studies the results are still controversial [6]. Ideally, the design and application of antioxidants must take into consideration the free radical or toxic compound target organ, the blood brain barrier (BBB) permeability of the compound, and the dose and time of administration [7]. In all cases, understanding the mechanisms of action of a compound with antioxidant properties is crucial to the design and implementation of clinical trials, which eventually will lead to more effective antioxidant therapeutics [6].

Glutathione and melatonin have been evaluated as protective compounds for the CNS. In the case of GSH, the protective effect is achieved by supplying glutathione's precursor amino acids, mainly cysteine. GSH does not cross the BBB, and oral or intravenous administration of GSH results in quick degradation or elimination. Several compounds have been tested to increase GSH in brain, for example the GSH monoethyl ester (GEE), L-2oxothiazolidine-carboxilic acid (OTC) and N-acetyl cysteine (NAC) [8]. Although in some cases GSH levels increased selectively in the brain (NAC) or specifically in astrocytes (OTC), either toxic ef-

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fects related to the compound or a lack of response in the target organ were observed.

More than a decade ago, the antioxidant properties of melatonin were described. This product of the pineal gland might exert its antioxidant effects directly by acting as a free radical scavenger and indirectly by increasing the expression and activity of antioxidants and decreasing the expression of pro-oxidant enzymes [3, 9, 12]. However, the precise mechanisms of melatonin's antioxidant effects are not fully understood.

This brief review focuses on the mechanisms of regulation of GSH and GSH-related enzymes by melatonin in the CNS. Maintenance of GSH in the CNS involves the interaction of neurons and non-neuronal cells such as astrocytes; in this context, some considerations about regulatory mechanisms of melatonin are also discussed.

THE GSH SYSTEM IN THE CNS

GSH is synthesized *de novo* in two sequential ATP-dependent reactions: first, γ-glutamate cysteine ligase (γ-GCL) catalyzes a reaction between glutamate and cysteine to form the dipeptide γ-glutamylcysteine; second, GSH synthetase (GS) catalyzes a reaction between γ-glutamylcysteine and glycine to produce GSH. Maintenance of the reduced form of GSH occurs by the enzyme glutathione reductase (GSR), which reduces GSSG to produce two GSH molecules in an NADPH-dependent reaction [13]. The glutathione system is comprised of both the reduced (most abundant, GSH) and oxidized (GSSG) forms of GSH and the enzymes related to its synthesis, maintenance and metabolism. GSH is the most abundant antioxidant in cells, although concentrations vary among different tissues. In the brain, where GSH provides a critical mechanism of endogenous defense against free radicals, the GSH concentration is approximately 1-3 mM [14, 15]. Selective increases in GSSG that lead to changes in the ratio of GSH/GSSG and a high production of ROS are associated with the development and progression of neural degeneration in disorders such as Parkinson's, Huntington's, and Alzheimer's diseases and in cerebellar degeneration [2, 5, 14]. Functions of the GSH system are essential for cellular maintenance and survival. For example, as an antioxidant, GSH serves as a hydrogen donor for the elimination of the free radical by-products of metabolic pathways that regenerate other cellular antioxidants such as α-tocopherol, ascorbate or ubiquinones. In reactions mediated by glutathione peroxidases (GPx), GSH also reduces organic peroxide by-products of LPO [16, 17]. GSH also reacts with toxic compounds, either spontaneously or enzymatically, through glutathione S-transferases (GSTs) to form excretable GSH-conjugates [16, 18-20]. GSH is a cysteine reservoir because this amino acid in its free form is easily auto-oxidized, thereby producing free radicals which result in neurotoxic effects [21, 22]. Moreover, in recent years, GSH has been found to modulate cellular signaling through "glutathionylation" of key cysteines located in the active site or regulatory regions of proteins like kinases, phosphatases and transcription factors [23, 24] or through the modulation of ROS-sensitive signal transduction pathways following changes in the intracellular redox state [20, 25-27].

As mentioned above, GSH levels have been reported in the range of 1-3 mM [14, 26], and it has been demonstrated that GSH content is higher in astrocytes than in neurons [28]. *In vitro*, GSH is released by astrocytes, and then, through extracellular catabolism, amino acids are generated and used as precursors for neuronal GSH synthesis [29, 30]. GSH is exported from astrocytes to the extracellular space through the multidrug resistance protein 1 [15, 31-33]. Once there, GSH is catabolized by ectopeptidases like γ-glutamyl transpeptidase (γ-GGT) that are present in the external neuronal membrane [30]. GSH precursors are then taken up by neurons through different amino acid transporter systems. Moreover, uptake of GSH precursors might vary with neuronal age. For example, cortical immature neurons exclusively uptake cysteine via the xCT system [34] whereas mature neurons use the cysteine-permeable, Na^+-dependent glutamate transporter system X_{AG}^- [8, 30, 35], glutamate transporters [8, 36], and possibly specific transporters such as GlyT 1 and 2 for glycine [37, 38]. In the CNS, the intracellular GSH content correlates with γ-GCL mRNA levels and enzymatic activity, and the GSH concentration is variable among different brain regions following the descending order cortex > cerebellum > hippocampus > brain stem [39].

MELATONIN

Melatonin (5-methoxy-*N*-acetyltryptamine) is synthesized from serotonin. This indoleamine is synthesized from tryptophan through the following reactions: 5-hydroxylation, decarboxylation, N-acetylation and O-methylation. Alternative pathways for melatonin formation involve O-methylation of serotonin followed by N-acetylation of 5-methoxytryptamine or O-methylation of tryptophan, decarboxylation and N-acetylation [10]. In vertebrates, melatonin is produced predominantly by the pineal gland, but it has been well documented that melatonin production also occurs in extrapineal sites such as the retina, Harderian gland, gastrointestinal tract, bone marrow, platelets, and skin [10, 12, 40-42]. It is well-known that the synthesis and secretion of melatonin are markedly influenced by light-dark cycles: during daylight the synthesis and secretion of melatonin are reduced while in darkness synthesis increases [40]. Melatonin produced by the pineal gland is released into the cerebrospinal fluid and the systemic circulation where it exerts various biological actions upon reaching melatonin receptor-rich target tissues [12]. Melatonin and its metabolites formed in the kynuramine pathway (see [9] for review) have multiple beneficial effects including biological modulation of mood; sleep regulation; sexual behavior; and anti-inflammatory, chronobiological, immunomodulatory, and neuroendocrine actions (see [10-12, 43, 44] for detailed reviews).

MELATONIN AS AN ANTIOXIDANT

Melatonin is of great clinical interest due to its potential ability to diminish the damage caused by increased levels of free radicals, neuronal loss, and motor or cognitive dysfunctions in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, Huntington's chorea, and amyotrophic lateral sclerosis, and in ischemia-reperfusion injury and traumatic brain injury [3, 43, 45, 46]. The antioxidant properties of melatonin in biological systems include a

direct antioxidant effect, which involves scavenging of ROS to prevent oxidative damage to cellular structures including membrane lipids and DNA (see [3, 11, 47] for reviews), and an indirect antioxidant effect, in which melatonin regulates the transcription and activity of antioxidant enzymes [48]. For example, melatonin induces γ-GCL mediated by AP-1 *in vitro* [49] and enhances GPx and SOD activities [50-53]. Interestingly, the inhibition of melatonin by light also inhibits the increase of SOD and GPx in avian and rodent tissues, which indicates a coordinated circadian regulation [54-57]. Additionally, in pinealectomized rats, GPx activity over 24 h was lower and LPO was increased, supporting the hypothesis that melatonin enhances GPx activity [58].

EVIDENCE THAT GSH AND GSH-RELATED ENZYMES ARE REGULATED BY MELATONIN IN THE CNS

Through subcutaneus or intraperitoneal administration, melatonin easily crosses the BBB and is detectable at higher concentrations in the brain relative to blood content [59, 60]. An interesting pharmacokinetic property of melatonin is its distribution into distinct structures of the brain following exogenous administration (Table **1**). Melatonin has been detected in cortex, hippocampus, cerebellum, striatum, thalamus/hypothalamus, midbrain and medulla (see Table **1**). Additionally, in Table **1** we summarize some examples from the literature in which melatonin has a positive effect on the regulation of GSH and its related enzymes under different stress conditions. Nevertheless, an intriguing question concerns the mechanisms through which this positive regulation occurs in the brain, considering the extraordinary complexity of this organ. A general cellular model for the regulation of antioxidant genes by melatonin has been proposed by Rodriguez *et al*. [48]. This model suggests that the regulation of antioxidant genes occurs through the activation of receptors MT1/2 and subsequent modulation of protein kinases, such as PKA, PKC, and members of mitogen activated protein kinases family (MAPK), and modulation of transcription factors, such as RZR/ROR nuclear orphan receptor, CREB/ATF, nuclear factor κB (NF- κB), and AP-1, that finally lead to the modulation of antioxidant-related genes [48]. This interesting and generalized model for the regulation of the expression of antioxidant enzymes might include additional components depending on the specific tissue. In the CNS, the regulation of the GSH system involves cellular interactions between astrocytes and neurons [30, 61]. Additionally, GSH enzymes are differentially distributed among different brain regions; that distribution is reflected in differential GSH content among the regions. In the next section of this review, we discuss evidence of melatonin regulation of GSH in the CNS considering the system's two main enzymes: GPxs and γ-GCL.

GPxs REGULATION IN THE CNS: THE "CLASSIC" ANTIOXIDANT TARGETS OF MELATONIN

Glutathione peroxidases constitute a family of selenoproteins that contain a selenocysteine at the active site that is successively oxidized and reduced during catalytic cycles, conferring the antioxidant capacity to eliminate peroxides using GSH as the reducing substrate. There are five members of the selenium-containing GPx family in mammals: cytosolic GPx (cGPx, GPx1), gastrointestinal GPx (GI-GPx, GPx2), plasma GPx (pGPx, GPx3), phospholipid hydroperoxide GPx (PHGPx, GPx4), and GPx6 which is restricted to the human olfactory system [77]. These proteins are produced according to a hierarchy that is dependent on the Se supply and mRNA stabilization; the hierarchy probably reflects the biological importance of the GPx family members [77, 78]. Under selenium deficiency, cGPx mRNA and protein are rapidly degraded, whereas other GPxs remain stable (e.g., PHGPx) or increase (e.g., GI-GPx) [79-81].

Early studies, first in rat brain and shortly thereafter in chick tissue, correlated exogenous melatonin administration with an increase in GPx activity [50, 53]. Interestingly, most of the changes in GPx activity reported in the literature have been evaluated by a coupled reaction that detects global peroxidase activity using GSR and cumene hydroperoxide as a substrate [50, 55, 82]. This method is limited because specific analyses of enzymatic activity and mRNA expression have demonstrated that in the brain, the cGPx and PHGPx isoforms are expressed differentially and moreover may be implicated in different functions [83, 84]. The cGPx is the classic peroxidase that reduces H_2O_2 produced by O_2 dismutation [85], meanwhile PHGPx functions include the reduction of inorganic and organic peroxides – particularly phospholipid hydroperoxides – within membranes and lipoproteins, the regulation of apoptosis, and gene expression, among others (reviewed in [86, 87]). Moreover, in rats, cGPx and PHGPx are differentially modulated by age and brain region. Both enzymes have been detected in cerebrum, cerebellum, midbrain, and brain stem [83]. Additionally, cGPx predominantly localizes to reticular thalamic and red nuclei, cortex, dentate gyrus, and pontine nucleus; moderate cGPx levels were observed in caudate-putamen, septum nuclei, the diagonal band of Broca, hippocampus, thalamus, and hypothalamus of the mouse brain [85]. At the cellular level, high levels of cGPx have been reported in microglia, whereas low levels have been detected in neurons and astrocytes in mouse and human brains [85, 88, 89]. In contrast, PHGPx mRNA is expressed mainly in neurons during postnatal development in the cortex, hippocampus, and cerebellum; mRNA levels decrease gradually in the brain of adult rats [86]. Additionally, PHGPx expression in astroglial has been detected in both the immediate surrounding area of an injury and in astrocytes located in distant areas from the site of injury [86]. There are three PHGPx isoforms reported: cytosolic (c-PHGPx), mitochondrial (m-PHGPx), and nuclear (n-PHGPx, mainly expressed during spermatogenesis) [87], but in the brain, just c-PHGPx and m-PHGPx have been detected by protein immunoreactivity and mRNA analysis [86]. There is little information about transcriptional regulation of GPx, but studies suggest a role of SRC kinases; the transcription factor NF-κB [90]; and SP-1, NF-1, and Smad family members [91, 92] in the up-regulation of PHGPx. Clearly, further evaluation of the transcription factors involved in the regulation of cGPx and PHGPx by melatonin is warranted.

How is melatonin exerting its effects on the regulation of GPxs in the brain? As mentioned before, a limited supply of selenium influences the expression of GPxs according to a hierarchy, but this criteria is only valid in the case of cGPx expression because PHGPx remains unchanged in brain regions under Se deficiency conditions [83]. On the other

Table 1. GSH, GSH-Related Enzymes and Antioxidants Modulated by Melatonin in different Brain Structures during Oxidative Stress Conditions

Brain Structure	Animal Model	Condition/Insult	Antioxidant Evaluated	Insult Consequence	Melatonin Administration	Melatonin Effect	Reference
Cortex	Rat	Polychlorinated biphenyls	GSH, Cu/Zn SOD, Mn SOD, GPx	↓ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities, tissue damage	i.p.	↑ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities	[62, 63]
	Rat	Formaldehyde	SOD, GPx	↓ SOD, GPx activities. ↑ MDA, apoptosis	i.p.	↑ enzymes activities, ↓ MDA and apoptosis	[64]
	Rat	Homocysteine	GSH, GPx	↑ Lipid peroxidation (LPO)	i.p.	↑GSH level and GPx activity	[65]
	Rat	Ischemia/ Reperfusion	SOD, GSR	↓ glucose consumption, ↓SOD and GSR	i.p.	↑ glucose consumption, ↑ SOD and GSR	[66]
	Rat	Kainic Acid	GSH	↑ GSSG/GSH ratio, ↑GPx, neuronal damage	i.p.	↓ GSSG/GSH, ↑ GPx	[67]
	Rat	Melatonin	GPx, Cu/Zn-SOD, Mn-SOD	Acute and chronic exposure	i.p.	↑ mRNA in all antioxidant enzymes	[68]
Hippocampus	Rat	Polychlorinated biphenyls	GSH, Cu/Zn SOD, Mn SOD, GPx	↓ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities, tissue damage	i.p.	↑ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities	[62, 63]
	Rat	Ethanol	GPx	↑ LPO	i.p.	↑ GPx, ↓ LPO	[69]
	Rat	Homocysteine	GSH, GPx	↑ LPO	i.p.	↑GSH level and GPx activity	[65]
	Rat	Lead	GSH, SOD	↑ LPO, ↓ GSH ↓ SOD activity, ↑ lost of neuronal density	i Subcutaneus injection	↓ LPO, ↑ GSH ↑ SOD activity, ↓ lost of neuronal density	[70]
	Rat	KA	GSH	↓ GSH	i.p.	Partial restoration of GSH	[71]
Cerebellum	Rat	Polychlorinated biphenyls	GSH, Cu/Zn SOD, Mn SOD, GPx	↓ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities, tissue damage	i.p	↑ Cu/Zn SOD, Mn SOD, GPx mRNA expression and activities	[62, 63]
	Rat	Homocysteine	GSH, GPx	↑ LPO	i.p	↑GSH level and GPx activity	[65]
	Rat	Ischemia/ Reperfusion	SOD, GSR	↓ glucose consumption, ↓SOD and GSR	i.p	↑ glucose consumption, ↑ SOD and GSR	[66]
	Mouse	Acute gamma radiation	GSH	↑ LPO, ↓ GSH, ↓ lost Purkinje Cells	i.p	↓LPO, ↑ GSH, ↑ lost Purkinje Cells	[72]

(Table 1) contd….

Brain Structure	Animal Model	Condition/Insult	Antioxidant Evaluated	Insult Consequence	Melatonin Administration	Melatonin Effect	Reference
Striatum	Rat	Kainic Acid	GSH	↑ GSSG/GSH ratio, ↑GPx, neuronal damage	i.p	↓ GSSG/GSH	[67]
	Rat	Lead	GSH, SOD	↑ LPO, ↓ GSH ↓ SOD activity, ↑ lost of neuronal density	Subcutaneus injection	↓ LPO, ↑ GSH ↑ SOD activity, ↓ lost of neuronal density	[70]
	Mouse	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine	GSH, SOD	↓ GSH, ↑ SOD	i.p	↑ GSH, ↑↑ SOD	[73]
	Mouse	1-methyl-4-phenyl-pyridinium ion/ Buthionin sulfoximine	GSH/GSSG	↓ GSH, ↑GSSG/GSH	i.p	↓ GSSG/GSH	[74]
Thalamus/ Hypothalamus	Rat	Ischemia/ Reperfusion	SOD, GSR	↓ glucose consumption, ↓SOD and GSR	i.p.	↑ glucose consumption, ↑ SOD and GSR	[66]
	Rat	Phosphine	GSH, GPX, SOD	↓GSH, ↓GPX, ↑ SOD	i.p.	↑ GSH, ↑ GPx, ↑↑ SOD	[75]
Midbrain	Rat	Ischemia/ Reperfusion	SOD, GSR	↓ glucose consumption, ↓SOD and GSR	i.p.	↑ glucose consumption, ↑ SOD and GSR	[66]
	Rat	Rotenone	GSH, SOD, CAT	↓ GSH, ↑ $OH^{\cdot}$	Intranigral	↑ GSH, SOD, CAT	[76]
Medulla	Rat	Ischemia/ Reperfusion	SOD, GSR	↓ glucose consumption, ↓SOD and GSR	i.p.	↑ glucose consumption, ↑ SOD and GSR	[66]

hand, administration of sodium selenite (0.1 mg/kg i.p.) and melatonin (10 mg/kg i.p.) in a rat model of ischemia/reperfusion restore GSH levels and global activity of GPxs, thereby preventing neuronal injury [93]. Interestingly, selenium and melatonin provide cellular protection from ROS damage through similar mechanisms. For example, both melatonin and Se enhance the expression and activity of GPxs, but melatonin also acts directly as a free radical scavenger [11]. The stabilization of GPx mRNA levels that occurs with an adequate supply of Se is necessary for GPxs regulation. The stabilization of mRNA levels has also been observed following exogenous melatonin administration, which strongly supports a common regulatory mechanism of GPxs that leads to increased protein synthesis and finally to higher peroxidase activity [94]. However, how this stabilization in mRNA levels of cGPx or PHGPx isoforms is modulated by melatonin is still unknown.

γ-GCL EXPRESSION, INCREASES IN GSH LEVELS, AND MELATONIN IN THE CNS: POTENTIAL MECHANISMS

As shown in Table **1**, under conditions of oxidative stress, melatonin administration is associated with increases in mRNA expression and activity of GPx and other GSH-related enzymes in several brain regions. However, in some cases, a corresponding increase in GSH content was not detected; rather, GSH levels were either maintained or slightly elevated [70, 71]. A possible explanation for the limited GSH recovery could be that high GPx activity for ROS detoxification increases GSH consumption, thereby limiting the full recovery of the thiol level. This effect might depend on the nature of the toxic agent, the amount of GSH participating in the processes, and on the ability of a specific brain region to activate GSH synthesis. In this regard, there is scarce evidence concerning the effect of melatonin on the expression of brain γ-GCL mRNA. This issue is important because γ-GCL (which includes the catalytic and modifier subunits) catalyzes the rate-limiting enzyme in GSH synthesis [95, 96]. Under oxidative stress conditions (Table **1**), intraperitoneal administration of melatonin increases the GSH content in some brain regions. However, it has been demonstrated that GSH synthesis correlates with γ-GCL mRNA expression and activity [39]. Thus, it could be speculated that the increase in the GSH content after melatonin administration observed in these experiments could be due to a coordinated increase in γ-GCL expression and activity as previ-

ously observed [26, 61, 97]. In support of this observation, some clues have emerged from diverse lines of investigation. First, sequence analysis studies have led to the identification of regulatory elements in the promoter of γ-GCLc and γ-GCLm. Mulcahy and coworkers have reported that the γ-GCLc and γ-GCLm promoters contain regulatory elements for transcription factors such as AP-1, activator protein-2 (AP-2), Sp-1, NF-κB, and Nrf2 [98-105]. Second, *in vitro* experiments have positively associated melatonin with the modulation of these transcription factors. For example, in ECV304 human vascular endothelial cells, 1 μM melatonin induced the expression of γ-GCL and resulted in an increase in the GSH content at 24 h by a mechanism that is tentatively linked to the transcriptional activity of AP-1 and retinoid Z receptor/retinoid receptor-related orphan receptor alpha (RZR/RORalpha) [49]. With respect to the transcription factor Nrf2, recent evidence indicates that melatonin increases Nrf2 expression as well as antioxidant enzyme levels under conditions of oxidative stress and inflammation in different tissues *in vivo* [106-108]. Additionally, melatonin has been linked to the activation of all of the aforementioned transcription factors through activation of MAPK family pathways (i.e., $p38^{MAPK}$ and ERK1/2), which raises the possibility of a melatonin-mediated signal transduction pathway that leads to an increase in γ-GCL expression and GSH in the CNS [26, 104-105, 109]. In support of such a melatonin-mediated pathway, *in vitro* and *in vivo* studies have demonstrated a modulation by melatonin of MAPK signaling [110-113]. For example, neuroprotective brain pathways activated by acute exposure to melatonin include Raf/MEK/ERK/p90RSK [114] and phosphatidyl inositol-3 kinase/Akt signaling [115]. In the same study, chronic prophylactic treatment with melatonin increased ERK-1/-2 and/or JNK-1/-2 phosphorylation [115]. Taken together, these data suggest that in the brain, the antioxidant effects of melatonin could stimulate MAPK or Akt signal transduction pathways, potentially leading to the regulation of γ-GCL which is reflected in higher GSH content. A neuron-glial cell interaction-based model that incorporates melatonin's effects on GSH synthesis and γ-GCL expression and regulation is still needed to test these hypotheses.

REGULATION OF THE GSH SYSTEM BY MELATONIN AND THE NEURON-ASTROCYTE INTERACTION

It is important to emphasize that in the CNS, modulation of the GSH system by melatonin occurs in an interactive environment involving both neurons and glia, particularly astrocytes. As mentioned initially, a clear biochemical interaction between neurons and astrocytes contributes to the regulation of GSH levels. First, some studies suggest that GSH concentrations are higher in cultured astrocytes (16-25 nmol/mg of protein) than in neurons (< 1 nmol/mg of protein) [116]. Interestingly, both catalytic and regulatory subunit proteins of γ-GCL are present not only in astrocytes but also in dopaminergic neurons of the substantia nigra [117]. Nevertheless, these differential amounts of GSH could vary by species and/or could be due to the use of different brain areas for the preparation of primary cultures or to different culture conditions [118]. However, the general consensus is that total GSH content is higher in astrocytes than in neurons [28, 116, 119-121], which could partially explain the higher susceptibility of neurons to oxidative damage as compared to astrocytes.

As shown in Table **1**, exogenous melatonin administration induced similar responses with respect to the modulation of GSH and related enzymes in the brain regions evaluated. However, melatonin has differential protective effects in CNS tissues such as brain, total spinal cord, optic nerve, and spinal cord white matter; this last tissue is where the major effect has been observed [122]. In the brain of pinealectomized rats, treatment with 500 μg/kg of melatonin twice daily for ten days significantly increased the content of 5-hydroxyindoleacetic acid (5-HIAA) in striatum and the 5-HIAA/5-HT ratio (an index of serotonergic activity) in nucleus accumbens. Interestingly, this evidence pinpoints the differential effect of melatonin in some brain regions [123].

Finally, it is unclear whether the neuron-astrocyte interaction is the same with respect to GSH synthesis in the presence or absence of melatonin. Some evidence demonstrates that melatonin exerts its neuroprotection by modulating this interaction. In a model of excitotoxicity induced with kainic acid in the hippocampus of mice, activation of neuronal Akt in hippocampal neurons and an up-regulation in the expression of astroglial cell line-derived neurotrophic factor (GDNF) were detected. That protection occurred by a direct effect of on astroglial GDNF levels, which subsequently activated the neuronal PI3K/Akt pathway [124]. As mentioned above, the PI3K/Akt pathway may be involved in neuroprotective responses in the brain [115]. Additionally, *in vitro* models strongly suggest that an increase of γ-GCL mRNA and GSH synthesis occur through activation of the PI3K/Akt/Nrf2 pathway [125-128]. Whether melatonin increases the GSH content and the expression of γ-GCL through PI3K/Akt/Nrf2 in astrocytes and neurons remains to be tested.

FINAL CONSIDERATIONS

The GSH system constitutes the main defense against oxidative damage. Disease, aging and environmental exposure through diet, pollution, etc. consume endogenous GSH and antioxidants increasing the risk for oxidative stress damage [4]. Melatonin is present in organisms at a concentration (< 1 nM) several orders of magnitude below that at which antioxidant effects have been reported both *in vitro* and *in vivo* [129], suggesting that the neurohormone is acting mainly as an antioxidant that quenches and/or reduces free radicals, although the activation of some signaling pathways might be expected in direct relationship to melatonin and melatonin receptor levels in the CNS. We have discussed evidence that exogenous melatonin can also exerts its protection by up regulating the GSH system. Additionally, it will be important to investigate the differential regional response to the presence of high levels of the neurohormone and to investigate if metabolic enzymes such as P450 cytochrome 1B1 (CYP1B1), which hydroxylates melatonin [130], are also modulated by its presence. Such modulation occurs with cytochromes from the 1A and 1B subfamilies that are known to be induced in response to environmental carcinogens such as polycyclic aromatic hydrocarbons (PAH, ubiquitous compounds present in urban air pollution and cigarette smoke).

This interaction could prove to be more complex than expected since melatonin protected the oxidative damage in brain by benzo(a)pirene, a carcinogenic PAH that consumes GSH [4]. The neuroprotection was observed as a significant inhibition of lipid peroxidation and induction of some enzymes of the GSH system like GPx and of SOD and CAT activities [131]. A similar response was observed in rat hippocampus where the administration of the neurohormone exerted protection against the pro-oxidant activity of aluminum lactate [132] and according to Riter *et al.* [133] melatonin protects against free radical-mediated damage by a wide variety of environmental insults. On the contrary, there is evidence that melatonin exacerbated the long-term potentiation impairment, learning and memory deficit induced by lead acetate [134]. This information is important due to the fact that diet supplementation with melatonin is advertised commercially as having antioxidant and neuroprotective effects, and its unregulated consumption could lead to toxic drug interactions.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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LIST OF ABBREVIATIONS

Akt	=	Alpha serine/threonine-protein kinase
AP-1	=	Activating Protein 1
AP-2	=	Activator protein-2
ATF	=	Activating transcription factor
BBB	=	Blood brain barrier
CAT	=	Catalase
CNS	=	Central nervous system
CREB	=	Cyclic-AMP response element binding protein
cGPx	=	Cytosolic GPx
CYP1B1	=	P450 cytochrome 1B1
ERK	=	Extracellular Signal-regulated Kinase
γ-GCL	=	Gamma-glutamate cysteine ligase
γ-GCLc	=	Gamma-glutamate cysteine ligase catalitic subunit
γ-GCLm	=	Gamma-glutamate cysteine ligase modifier subunit
γ-GGT	=	Gamma glutamyl transpeptidase
GS	=	Glutathione synthetase
GPxs	=	Glutathione peroxidases
GSTs	=	Glutathione S-transferases
GDNF	=	Astroglial cell line-derived neurotrophic factor
GEE	=	GSH monoethyl ester
GI-GPx	=	Gastrointestinal GPx
GSR	=	Glutathione Reductase
GSH	=	Reduced Glutathione
GSSG	=	Oxidized Glutathione
GlyT 1 and 2	=	Glycine transporters 1 and 2
5-HT	=	5-hydroxytriptamine
5-HIAA	=	5-hydroxyindoleacetic acid
JNK,	=	c-Jun amino N-terminal kinase
MAPK	=	Mitogen activated protein Kinase
MEK	=	MAPK/ERK kinase
NAC	=	N-acetyl cysteine
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NF-1	=	Neurofibromin 1
NF-kB	=	Nuclear factor kappa B
Nrf2	=	Nuclear factor E2 p45-related factor
OTC	=	L-2oxothiazolidine-carboxilic acid
$p38^{MAPK}$	=	p38 kinase
p90RSK	=	Ribosomal protein S6 kinase
PHGPx	=	Phospholipid hydroperoxide GPx
PI3K	=	Phosphatidylinositol 3-kinase
PKA	=	Protein kinase A
PKC	=	Protein kinase C
PAH	=	Polycyclic aromatic hydrocarbons
Raf	=	Proto-oncogene serine/threonine-protein kinase
ROS	=	Reactive oxygen species
RZR/ROR	=	Nuclear hormone orphan receptor
SOD	=	Superoxide dismutase
SP-1	=	Sex-specific storage-protein 1
SRC	=	Sarcoma tyrosine kinases
xCT	=	Subunit of cysteine transporter
X_{AG}^-	=	Cysteine-permeable, Na^+-dependent glutamate transporter

REFERENCES

[1] Lee, D.W.; Andersen, J.K. Iron elevations in the aging Parkinsonian brain: a consequence of impaired iron homeostasis? *J. Neurochem.*, **2010**, *112*(2), 332-339.

[2] Bains, J.S.; Shaw, C.A. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res. Brain Res. Rev.*, **1997**, *25*(3), 335-358.

[3] Reiter, R.J. Oxidative damage in the central nervous system: protection by melatonin. *Prog. Neurobiol.*, **1998**, *56*(3), 359-384.

[4] Limon-Pacheco, J.; Gonsebatt, M.E. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. *Mutat. Res.*, **2009**, *674*(1-2), 137-147.

[5] Limón-Pacheco, J.H.; Gonsebatt M.E. In *New Perspectives on Brain Cell Damage, Neurodegeneration and Neuroprotective Strategies*. Santamaría, A.; Jiménez-Capdeville, .M.E. Ed.; Research Signpost: Trivandrum, **2007**; pp 173-188.

[6] Kamat, C.D.; Gadal, S.; Mhatre, M.; Williamson, K.S.; Pye, Q.N.; Hensley, K. Antioxidants in central nervous system diseases: preclinical promise and translational challenges. *J. Alzheimers Dis.*, **2008**, *15*(3), 473-493.

[7] Gilgun-Sherki, Y.; Rosenbaum, Z.; Melamed, E.; Offen, D. Antioxidant therapy in acute central nervous system injury: current state. *Pharmacol. Rev.*, **2002**, *54*(2), 271-284.

[8] Aoyama, K.; Watabe, M.; Nakaki, T. Regulation of neuronal glutathione synthesis. *J. Pharmacol. Sci.*, **2008**, *108*(3), 227-238.

[9] Hardeland, R. Neuroprotection by radical avoidance: search for suitable agents. *Molecules*, **2009**, *14*(12), 5054-5102.

[10] Hardeland, R.; Pandi-Perumal, S.R.; Cardinali, D.P. Melatonin. *Int. J. Biochem. Cell Biol.*, **2006**, *38*(3), 313-316.

[11] Reiter, R.J.; Acuna-Castroviejo, D.; Tan, D.X.; Burkhardt, S. Free radical-mediated molecular damage: mechanisms for the protective actions of melatonin in the central nervous system. *Ann. N. Y. Acad. Sci.*, **2001**, *939*, 200-215.

[12] Zawilska, J.B.; Skene, D.J.; Arendt, J. Physiology and pharmacology of melatonin in relation to biological rhythms. *Pharmacol. Rep.*, **2009**, *61*(3), 383-410.

[13] Meister, A. Metabolism and function of glutathione: an overview. *Biochem. Soc. Trans.*, **1982**, *10*(2), 78-79.

[14] Dringen, R. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.*, **2000**, *62*(6), 649-671.

[15] Dringen, R.; Hirrlinger, J. Glutathione pathways in the brain. *Biol. Chem.*, **2003**, *384*(4), 505-516.

[16] Anderson, M.E. Glutathione: an overview of biosynthesis and modulation. *Chem. Biol. Interact.*, **1998**, *111-112*, 1-14.

[17] Dringen, R.; Gutterer, J.M.; Hirrlinger, J. Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. *Eur. J. Biochem.*, **2000**, *267*(16), 4912-4916.

[18] Ketterer, B.; Coles, B.; Meyer, D.J. The role of glutathione in detoxication. *Environ. Health Perspect.*, **1983**, *49*, 59-69.

[19] Monks, T.J.; Lau, S.S. Glutathione conjugation as a mechanism for the transport of reactive metabolites. *Adv. Pharmacol.*, **1994**, *27*, 183-210.

[20] Strange, R.C.; Jones, P.W.; Fryer, A.A. Glutathione S-transferase: genetics and role in toxicology. *Toxicol. Lett.*, **2000**, *112-113*, 357-363.

[21] Janaky, R.; Varga, V.; Hermann, A.; Saransaari, P.; Oja, S.S. Mechanisms of L-cysteine neurotoxicity. *Neurochem. Res.*, **2000**, *25*(9-10), 1397-1405.

[22] Lu, S.C. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J.*, **1999**, *13*(10), 1169-1183.

[23] Anselmo, A.N.; Cobb, M.H. Protein kinase function and glutathionylation. *Biochem. J.*, **2004**, *381*(Pt 3), e1-2.

[24] Reynaert, N.L.; van der Vliet, A.; Guala, A.S.; McGovern, T.; Hristova, M.; Pantano, C.; Heintz, N.H.; Heim, J.; Ho, Y.S.; Matthews, D.E.; Wouters, E.F.; Janssen-Heininger, Y.M. Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*(35), 13086-13091.

[25] Cross, J.V.; Templeton, D.J. Regulation of signal transduction through protein cysteine oxidation. *Antioxid. Redox Signal.*, **2006**, *8*(9-10), 1819-1827.

[26] Limon-Pacheco, J.H.; Hernandez, N.A.; Fanjul-Moles, M.L.; Gonsebatt, M.E. Glutathione depletion activates mitogen-activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice. *Free Radic. Biol. Med.*, **2007**, *43*(9), 1335-1347.

[27] Winterbourn, C.C.; Hampton, M.B. Thiol chemistry and specificity in redox signaling. *Free Radic. Biol. Med.*, **2008**, *45*(5), 549-561.

[28] Rice, M.E.; Russo-Menna, I. Differential compartmentalization of brain ascorbate and glutathione between neurons and glia. *Neuroscience*, **1998**, *82*(4), 1213-1223.

[29] Dringen, R.; Pfeiffer, B.; Hamprecht, B. Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J. Neurosci.*, **1999**, *19*(2), 562-569.

[30] Shanker, G.; Allen, J.W.; Mutkus, L.A.; Aschner, M. The uptake of cysteine in cultured primary astrocytes and neurons. *Brain Res.*, **2001**, *902*(2), 156-163.

[31] Hirrlinger, J.; Konig, J.; Keppler, D.; Lindenau, J.; Schulz, J.B.; Dringen, R. The multidrug resistance protein MRP1 mediates the release of glutathione disulfide from rat astrocytes during oxidative stress. *J. Neurochem.*, **2001**, *76*(2), 627-636.

[32] Hirrlinger, J.; Schulz, J.B.; Dringen, R. Glutathione release from cultured brain cells: multidrug resistance protein 1 mediates the release of GSH from rat astroglial cells. *J. Neurosci. Res.*, **2002**, *69*(3), 318-326.

[33] Minich, T.; Riemer, J.; Schulz, J.B.; Wielinga, P.; Wijnholds, J.; Dringen, R. The multidrug resistance protein 1 (Mrp1), but not Mrp5, mediates export of glutathione and glutathione disulfide from brain astrocytes. *J. Neurochem.*, **2006**, *97*(2), 373-384.

[34] Murphy, T.H.; Schnaar, R.L.; Coyle, J.T. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. *FASEB J.*, **1990**, *4*(6), 1624-1633.

[35] Chen, Y.; Swanson, R.A. The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures. *J. Neurochem.*, **2003**, *84*(6), 1332-1339.

[36] Watabe, M.; Aoyama, K.; Nakaki, T. A dominant role of GTRAP3-18 in neuronal glutathione synthesis. *J. Neurosci.*, **2008**, *28*(38), 9404-9413.

[37] Zafra, F.; Aragon, C.; Olivares, L.; Danbolt, N.C.; Gimenez, C.; Storm-Mathisen, J. Glycine transporters are differentially expressed among CNS cells. *J. Neurosci.*, **1995**, *15*(5 Pt 2), 3952-3969.

[38] Zafra, F.; Gomeza, J.; Olivares, L.; Aragon, C.; Gimenez, C. Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. *Eur. J. Neurosci.*, **1995**, *7*(6), 1342-1352.

[39] Kang, Y.; Viswanath, V.; Jha, N.; Qiao, X.; Mo, J.Q.; Andersen, J.K. Brain gamma-glutamyl cysteine synthetase (GCS) mRNA expression patterns correlate with regional-specific enzyme activities and glutathione levels. *J. Neurosci. Res.*, **1999**, *58*(3), 436-441.

[40] Chattoraj, A.; Liu, T.; Zhang, L.S.; Huang, Z.; Borjigin, J. Melatonin formation in mammals: *in vivo* perspectives. *Rev. Endocr. Metab. Disord.*, **2009**, *10*(4), 237-243.

[41] Reiter, R.J.; Tan, D.X.; Korkmaz, A. The circadian melatonin rhythm and its modulation: possible impact on hypertension. *J. Hypertens. Suppl.*, **2009**, *27*(6), S17-20.

[42] Tan, D.X.; Hardeland, R.; Manchester, L.C.; Paredes, S.D.; Korkmaz, A.; Sainz, R.M.; Mayo, J.C.; Fuentes-Broto, L.; Reiter, R.J. The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.*, **2009**, doi: 10.1111/j.1469-185X.2009.00118.x.

[43] Kaur, C. Sivakumar, V. Ling, E. A. Melatonin and its Therapeutic potential in neuroprotection. *Central Nervous Syst. Agents Med. Chem.*, **2008**, *8*(4), 260-266.

[44] Poeggeler, B. Melatonin, aging, and age-related diseases: perspectives for prevention, intervention, and therapy. *Endocrine*, **2005**, *27*(2), 201-212.

[45] Mayo, J.C.; Sainz, R.M.; Tan, D.X.; Antolin, I.; Rodriguez, C.; Reiter, R.J. Melatonin and Parkinson's disease. *Endocrine*, **2005**, *27*(2), 169-178.

[46] Tan, D.X.; Manchester, L.C.; Sainz, R.M.; Mayo, J.C.; Leon, J.; Reiter, R.J. Physiological ischemia/reperfusion phenomena and their relation to endogenous melatonin production: a hypothesis. *Endocrine*, **2005**, *27*(2), 149-158.

[47] Reiter, R.J. Cytoprotective properties of melatonin: presumed association with oxidative damage and aging. *Nutrition*, **1998**, *14*(9), 691-696.

[48] Rodriguez, C.; Mayo, J.C.; Sainz, R.M.; Antolin, I.; Herrera, F.; Martin, V.; Reiter, R.J. Regulation of antioxidant enzymes: a significant role for melatonin. *J. Pineal. Res.*, **2004**, *36*(1), 1-9.

[49] Urata, Y.; Honma, S.; Goto, S.; Todoroki, S.; Iida, T.; Cho, S.; Honma, K.; Kondo, T. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic. Biol. Med.*, **1999**, *27*(7-8), 838-847.

[50] Barlow-Walden, L.R.; Reiter, R.J.; Abe, M.; Pablos, M.; Menendez-Pelaez, A.; Chen, L.D.; Poeggeler, B. Melatonin stimulates brain glutathione peroxidase activity. *Neurochem. Int.*, **1995**, *26*(5), 497-502.

[51] Liu, F.; Ng, T.B. Effect of pineal indoles on activities of the antioxidant defense enzymes superoxide dismutase, catalase, and glutathione reductase, and levels of reduced and oxidized glutathione in rat tissues. *Biochem. Cell Biol.*, **2000**, *78*(4), 447-453.

[52] Ozturk, G.; Coskun, S.; Erbas, D.; Hasanoglu, E. The effect of melatonin on liver superoxide dismutase activity, serum nitrate and thyroid hormone levels. *Jpn. J. Physiol.*, **2000**, *50*(1), 149-153.

[53] Pablos, M.I.; Agapito, M.T.; Gutierrez, R.; Recio, J.M.; Reiter, R.J.; Barlow-Walden, L.; Acuna-Castroviejo, D.; Menendez-Pelaez, A. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J. Pineal Res.*, **1995**, *19*(3), 111-115.

[54] Albarran, M.T.; Lopez-Burillo, S.; Pablos, M.I.; Reiter, R.J.; Agapito, M.T. Endogenous rhythms of melatonin, total antioxidant status and superoxide dismutase activity in several tissues of chick and their inhibition by light. *J. Pineal Res.*, **2001**, *30*(4), 227-233.

[55] Pablos, M.I.; Reiter, R.J.; Ortiz, G.G.; Guerrero, J.M.; Agapito, M.T.; Chuang, J.I.; Sewerynek, E. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. *Neurochem. Int.*, **1998**, *32*(1), 69-75.

[56] Tomas-Zapico, C.; Coto-Montes, A.; Martinez-Fraga, J.; Rodriguez-Colunga, M.J.; Tolivia, D. Effects of continuous light exposure on antioxidant enzymes, porphyric enzymes and cellular damage in the Harderian gland of the Syrian hamster. *J. Pineal Res.*, **2003**, *34*(1), 60-68.

[57] Tunez, I.; del Carmen Munoz, M.; Feijoo, M.; Valdelvira, M.E.; Rafael Munoz-Castaneda, J.; Montilla, P. Melatonin effect on renal oxidative stress under constant light exposure. *Cell Biochem. Funct.*, **2003**, *21*(1), 35-40.

[58] Baydas, G.; Gursu, M.F.; Yilmaz, S.; Canpolat, S.; Yasar, A.; Cikim, G.; Canatan, H. Daily rhythm of glutathione peroxidase activity, lipid peroxidation and glutathione levels in tissues of pinealectomized rats. *Neurosci. Lett.*, **2002**, *323*(3), 195-198.

[59] Menendez-Pelaez, A.; Poeggeler, B.; Reiter, R.J.; Barlow-Walden, L.; Pablos, M.I.; Tan, D.X. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. *J. Cell. Biochem.*, **1993**, *53*(4), 373-382.

[60] Menendez-Pelaez, A.; Reiter, R.J. Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. *J. Pineal Res.*, **1993**, *15*(2), 59-69.

[61] Shih, A.Y.; Johnson, D.A.; Wong, G.; Kraft, A.D.; Jiang, L.; Erb, H.; Johnson, J.A.; Murphy, T.H. Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J. Neurosci.*, **2003**, *23*(8), 3394-3406.

[62] Venkataraman, P.; Krishnamoorthy, G.; Vengatesh, G.; Srinivasan, N.; Aruldhas, M.M.; Arunakaran, J. Protective role of melatonin on PCB (Aroclor 1,254) induced oxidative stress and changes in acetylcholine esterase and membrane bound ATPases in cerebellum, cerebral cortex and hippocampus of adult rat brain. *Int. J. Dev. Neurosci.*, **2008**, *26*(6), 585-591.

[63] Venkataraman, P.; Selvakumar, K.; Krishnamoorthy, G.; Muthusami, S.; Rameshkumar, R.; Prakash, S.; Arunakaran, J. Effect of melatonin on PCB (Aroclor 1254) induced neuronal damage and changes in Cu/Zn superoxide dismutase and glutathione peroxidase-4 mRNA expression in cerebral cortex, cerebellum and hippocampus of adult rats. *Neurosci. Res.*, **2010**, *66*(2), 189-197.

[64] Zararsiz, I.; Kus, I.; Ogeturk, M.; Akpolat, N.; Kose, E.; Meydan, S.; Sarsilmaz, M. Melatonin prevents formaldehyde-induced neurotoxicity in prefrontal cortex of rats: an immunohistochemical and biochemical study. *Cell Biochem. Funct.*, **2007**, *25*(4), 413-418.

[65] Baydas, G.; Kutlu, S.; Naziroglu, M.; Canpolat, S.; Sandal, S.; Ozcan, M.; Kelestimur, H. Inhibitory effects of melatonin on neural lipid peroxidation induced by intracerebroventricularly administered homocysteine. *J. Pineal Res.*, **2003**, *34*(1), 36-39.

[66] El-Abhar, H.S.; Shaalan, M.; Barakat, M.; El-Denshary, E.S. Effect of melatonin and nifedipine on some antioxidant enzymes and different energy fuels in the blood and brain of global ischemic rats. *J. Pineal Res.*, **2002**, *33*(2), 87-94.

[67] Chen, S.T.; Chuang, J.I. The antioxidant melatonin reduces cortical neuronal death after intrastriatal injection of kainate in the rat. *Exp. Brain Res.*, **1999**, *124*(2), 241-247.

[68] Kotler, M.; Rodriguez, C.; Sainz, R.M.; Antolin, I.; Menendez-Pelaez, A. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J. Pineal Res.*, **1998**, *24*(2), 83-89.

[69] Gonenc, S.; Uysal, N.; Acikgoz, O.; Kayatekin, B.M.; Sonmez, A.; Kiray, M.; Aksu, I.; Gulecer, B.; Topcu, A.; Semin, I. Effects of melatonin on oxidative stress and spatial memory impairment induced by acute ethanol treatment in rats. *Physiol. Res.*, **2005**, *54*(3), 341-348.

[70] El-Sokkary, G.H.; Kamel, E.S.; Reiter, R.J. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. *Cell. Mol. Biol. Lett.*, **2003**, *8*(2), 461-470.

[71] Floreani, M.; Skaper, S.D.; Facci, L.; Lipartiti, M.; Giusti, P. Melatonin maintains glutathione homeostasis in kainic acid-exposed rat brain tissues. *FASEB J.*, **1997**, *11*(14), 1309-1315.

[72] Sisodia, R.; Kumari, S.; Verma, R.K.; Bhatia, A.L. Prophylactic role of melatonin against radiation induced damage in mouse cerebellum with special reference to Purkinje cells. *J. Radiol. Prot.*, **2006**, *26*(2), 227-234.

[73] Thomas, B.; Mohanakumar, K.P. Melatonin protects against oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the mouse nigrostriatum. *J. Pineal Res.*, **2004**, *36*(1), 25-32.

[74] Chen, S.T.; Chuang, J.I.; Hong, M.H.; Li, E.I. Melatonin attenuates MPP+-induced neurodegeneration and glutathione impairment in the nigrostriatal dopaminergic pathway. *J. Pineal Res.*, **2002**, *32*(4), 262-269.

[75] Hsu, C.H.; Chi, B.C.; Casida, J.E. Melatonin reduces phosphine-induced lipid and DNA oxidation *in vitro* and *in vivo* in rat brain. *J. Pineal Res.*, **2002**, *32*(1), 53-58.

[76] Saravanan, K.S.; Sindhu, K.M.; Mohanakumar, K.P. Melatonin protects against rotenone-induced oxidative stress in a hemiparkinsonian rat model. *J. Pineal Res.*, **2007**, *42*(3), 247-253.

[77] Brigelius-Flohe, R. Glutathione peroxidases and redox-regulated transcription factors. *Biol. Chem.*, **2006**, *387*(10-11), 1329-1335.

[78] Muller, C.; Wingler, K.; Brigelius-Flohe, R. 3'UTRs of glutathione peroxidases differentially affect selenium-dependent mRNA stability and selenocysteine incorporation efficiency. *Biol. Chem.*, **2003**, *384*(1), 11-18.

[79] Bermano, G.; Nicol, F.; Dyer, J.A.; Sunde, R.A.; Beckett, G.J.; Arthur, J.R.; Hesketh, J.E. Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochem. J.*, **1995**, *311* (Pt 2), 425-430.

[80] Lei, X.G.; Evenson, J.K.; Thompson, K.M.; Sunde, R.A. Glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase are differentially regulated in rats by dietary selenium. *J. Nutr.*, **1995**, *125*(6), 1438-1446.

[81] Wingler, K.; Bocher, M.; Flohe, L.; Kollmus, H.; Brigelius-Flohe, R. mRNA stability and selenocysteine insertion sequence efficiency rank gastrointestinal glutathione peroxidase high in the hierarchy of selenoproteins. *Eur. J. Biochem.*, **1999**, *259*(1-2), 149-157.

[82] Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, **1967**, *70*(1), 158-169.

[83] Mitchell, J.H.; Nicol, F.; Beckett, G.J.; Arthur, J.R. Selenium and iodine deficiencies: effects on brain and brown adipose tissue selenoenzyme activity and expression. *J. Endocrinol.*, **1997**, *155*(2), 255-263.

[84] Zhang, L.P.; Maiorino, M.; Roveri, A.; Ursini, F. Phospholipid hydroperoxide glutathione peroxidase: specific activity in tissues of rats of different age and comparison with other glutathione peroxidases. *Biochim. Biophys. Acta*, **1989**, *1006*(1), 140-143.

[85] Trepanier, G.; Furling, D.; Puymirat, J.; Mirault, M.E. Immunocytochemical localization of seleno-glutathione peroxidase in the adult mouse brain. *Neuroscience*, **1996**, *75*(1), 231-243.

[86] Savaskan, N.E.; Borchert, A.; Brauer, A.U.; Kuhn, H. Role for glutathione peroxidase-4 in brain development and neuronal apoptosis: specific induction of enzyme expression in reactive astrocytes following brain injury. *Free Radic. Biol. Med.*, **2007**, *43*(2), 191-201.

[87] Savaskan, N.E.; Ufer, C.; Kuhn, H.; Borchert, A. Molecular biology of glutathione peroxidase 4: from genomic structure to developmental expression and neural function. *Biol. Chem.*, **2007**, *388*(10), 1007-1017.

[88] Lindenau, J.; Noack, H.; Asayama, K.; Wolf, G. Enhanced cellular glutathione peroxidase immunoreactivity in activated astrocytes

and in microglia during excitotoxin induced neurodegeneration. *Glia*, **1998**, *24*(2), 252-256.

[89] Power, J.H.; Blumbergs, P.C. Cellular glutathione peroxidase in human brain: cellular distribution, and its potential role in the degradation of Lewy bodies in Parkinson's disease and dementia with Lewy bodies. *Acta Neuropathol.*, **2009**, *117*(1), 63-73.

[90] Hattori, H.; Imai, H.; Furuhama, K.; Sato, O.; Nakagawa, Y. Induction of phospholipid hydroperoxide glutathione peroxidase in human polymorphonuclear neutrophils and HL60 cells stimulated with TNF-alpha. *Biochem. Biophys. Res. Commun.*, **2005**, *337*(2), 464-473.

[91] Huang, H.S.; Chen, C.J.; Chang, W.C. The CCAAT-box binding factor NF-Y is required for the expression of phospholipid hydroperoxide glutathione peroxidase in human epidermoid carcinoma A431 cells. *FEBS Lett.*, **1999**, *455*(1-2), 111-116.

[92] Ufer, C.; Borchert, A.; Kuhn, H. Functional characterization of cis- and trans-regulatory elements involved in expression of phospholipid hydroperoxide glutathione peroxidase. *Nucleic Acids Res.*, **2003**, *31*(15), 4293-4303.

[93] Ahmad, A.; Khan, M.M.; Ishrat, T.; Khan, M.B.; Khuwaja, G.; Raza, S.S.; Shrivastava, P.; Islam, F. Synergistic effect of selenium and melatonin on neuroprotection in cerebral ischemia in rats. *Biol. Trace Elem. Res.*, **2010**, [Epub ahead of Print].

[94] Mayo, J.C.; Sainz, R.M.; Antoli, I.; Herrera, F.; Martin, V.; Rodriguez, C. Melatonin regulation of antioxidant enzyme gene expression. *Cell. Mol. Life Sci.*, **2002**, *59*(10), 1706-1713.

[95] Dickinson, D.A.; Levonen, A.L.; Moellering, D.R.; Arnold, E.K.; Zhang, H.; Darley-Usmar, V.M.; Forman, H.J. Human glutamate cysteine ligase gene regulation through the electrophile response element. *Free Radic. Biol. Med.*, **2004**, *37*(8), 1152-1159.

[96] Krzywanski, D.M.; Dickinson, D.A.; Iles, K.E.; Wigley, A.F.; Franklin, C.C.; Liu, R.M.; Kavanagh, T.J.; Forman, H.J. Variable regulation of glutamate cysteine ligase subunit proteins affects glutathione biosynthesis in response to oxidative stress. *Arch. Biochem. Biophys.*, **2004**, *423*(1), 116-125.

[97] Sun, X.; Erb, H.; Murphy, T.H. Coordinate regulation of glutathione metabolism in astrocytes by Nrf2. *Biochem. Biophys. Res. Commun.*, **2005**, *326*(2), 371-377.

[98] Erickson, A.M.; Nevarea, Z.; Gipp, J.J.; Mulcahy, R.T. Identification of a variant antioxidant response element in the promoter of the human glutamate-cysteine ligase modifier subunit gene. Revision of the ARE consensus sequence. *J. Biol. Chem.*, **2002**, *277*(34), 30730-30737.

[99] Moinova, H.R.; Mulcahy, R.T. An electrophile responsive element (EpRE) regulates beta-naphthoflavone induction of the human gamma-glutamylcysteine synthetase regulatory subunit gene. Constitutive expression is mediated by an adjacent AP-1 site. *J. Biol. Chem.*, **1998**, *273*(24), 14683-14689.

[100] Mulcahy, R.T.; Gipp, J.J. Identification of a putative antioxidant response element in the 5'-flanking region of the human gamma-glutamylcysteine synthetase heavy subunit gene. *Biochem. Biophys. Res. Commun.*, **1995**, *209*(1), 227-233.

[101] Mulcahy, R.T.; Wartman, M.A.; Bailey, H.H.; Gipp, J.J. Constitutive and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. *J. Biol. Chem.*, **1997**, *272*(11), 7445-7454.

[102] Wild, A.C.; Gipp, J.J.; Mulcahy, T. Overlapping antioxidant response element and PMA response element sequences mediate basal and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase catalytic subunit gene. *Biochem. J.*, **1998**, *332* (Pt 2), 373-381.

[103] Wild, A.C.; Moinova, H.R.; Mulcahy, R.T. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J. Biol. Chem.*, **1999**, *274*(47), 33627-33636.

[104] Zipper, L.M.; Mulcahy, R.T. Inhibition of ERK and p38 MAP kinases inhibits binding of Nrf2 and induction of GCS genes. *Biochem. Biophys. Res. Commun.*, **2000**, *278*(2), 484-492.

[105] Zipper, L.M.; Mulcahy, R.T. Erk activation is required for Nrf2 nuclear localization during pyrrolidine dithiocarbamate induction of glutamate cysteine ligase modulatory gene expression in HepG2 cells. *Toxicol. Sci.*, **2003**, *73*(1), 124-134.

[106] Jung, K.H.; Hong, S.W.; Zheng, H.M.; Lee, D.H.; Hong, S.S. Melatonin downregulates nuclear erythroid 2-related factor 2 and nuclear factor-kappaB during prevention of oxidative liver injury in a dimethylnitrosamine model. *J. Pineal Res.*, **2009**, *47*(2), 173-183.

[107] Jung, K.H.; Hong, S.W.; Zheng, H.M.; Lee, H.S.; Lee, H.; Lee, D.H.; Lee, S.Y.; Hong, S.S. Melatonin ameliorates cerulein-induced pancreatitis by the modulation of nuclear erythroid 2-related factor 2 and nuclear factor-kappaB in rats. *J. Pineal Res.*, **2010**, *48*(3), 239-250.

[108] Tripathi, D.N.; Jena, G.B. Effect of melatonin on the expression of Nrf2 and NF-kappaB during cyclophosphamide-induced urinary bladder injury in rat. *J. Pineal Res.*, **2010**, *48*(4), 324-331.

[109] Shih, P.H.; Yen, G.C. Differential expressions of antioxidant status in aging rats: the role of transcriptional factor Nrf2 and MAPK signaling pathway. *Biogerontology*, **2007**, *8*(2), 71-80.

[110] Esposito, E.; Genovese, T.; Caminiti, R.; Bramanti, P.; Meli, R.; Cuzzocrea, S. Melatonin reduces stress-activated/mitogen-activated protein kinases in spinal cord injury. *J. Pineal Res.*, **2009**, *46*(1), 79-86.

[111] Kimball, S.R.; Abbas, A.; Jefferson, L.S. Melatonin represses oxidative stress-induced activation of the MAP kinase and mTOR signaling pathways in H4IIE hepatoma cells through inhibition of Ras. *J. Pineal Res.*, **2008**, *44*(4), 379-386.

[112] Luchetti, F.; Betti, M.; Canonico, B.; Arcangeletti, M.; Ferri, P.; Galli, F.; Papa, S. ERK MAPK activation mediates the antiapoptotic signaling of melatonin in UVB-stressed U937 cells. *Free Radic. Biol. Med.*, **2009**, *46*(3), 339-351.

[113] Zhu, H.Q.; Cheng, X.W.; Xiao, L.L.; Jiang, Z.K.; Zhou, Q.; Gui, S.Y.; Wei, W.; Wang, Y. Melatonin prevents oxidized low-density lipoprotein-induced increase of myosin light chain kinase activation and expression in HUVEC through ERK/MAPK signal transduction. *J. Pineal Res.*, **2008**, *45*(3), 328-334.

[114] Koh, P.O. Melatonin attenuates the cerebral ischemic injury via the MEK/ERK/p90RSK/bad signaling cascade. *J. Vet. Med. Sci.*, **2008**, *70*(11), 1219-1223.

[115] Kilic, U.; Kilic, E.; Reiter, R.J.; Bassetti, C.L.; Hermann, D.M. Signal transduction pathways involved in melatonin-induced neuroprotection after focal cerebral ischemia in mice. *J. Pineal. Res.*, **2005**, *38*(1), 67-71.

[116] Raps, S.P.; Lai, J.C.; Hertz, L.; Cooper, A.J. Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. *Brain Res.*, **1989**, *493*(2), 398-401.

[117] Yantiri, F.; Gasparyan, A.; Andersen, J.K. Glutamyl cysteine synthetase catalytic and regulatory subunits localize to dopaminergic nigral neurons as well as to astrocytes. *J. Neurosci. Res.*, **2001**, *64*(2), 203-206.

[118] Pileblad, E.; Eriksson, P.S.; Hansson, E. The presence of glutathione in primary neuronal and astroglial cultures from rat cerebral cortex and brain stem. *J. Neural Transm. Gen. Sect.*, **1991**, *86*(1), 43-49.

[119] Dringen, R.; Kussmaul, L.; Gutterer, J.M.; Hirrlinger, J.; Hamprecht, B. The glutathione system of peroxide detoxification is less efficient in neurons than in astroglial cells. *J. Neurochem.*, **1999**, *72*(6), 2523-2530.

[120] Huang, J.; Philbert, M.A. Distribution of glutathione and glutathione-related enzyme systems in mitochondria and cytosol of cultured cerebellar astrocytes and granule cells. *Brain Res.*, **1995**, *680*(1-2), 16-22.

[121] Makar, T.K.; Nedergaard, M.; Preuss, A.; Gelbard, A.S.; Perumal, A.S.; Cooper, A.J. Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glutathione metabolism in cultures of chick astrocytes and neurons: evidence that astrocytes play an important role in antioxidative processes in the brain. *J. Neurochem.*, **1994**, *62*(1), 45-53.

[122] Kaptanoglu, E.; Palaoglu, S.; Demirpence, E.; Akbiyik, F.; Solaroglu, I.; Kilinc, A. Different responsiveness of central nervous system tissues to oxidative conditions and to the antioxidant effect of melatonin. *J. Pineal Res.*, **2003**, *34*(1), 32-35.

[123] Miguez, J.M.; Martin, F.J.; Aldegunde, M. Melatonin effects on serotonin synthesis and metabolism in the striatum, nucleus accumbens, and dorsal and median raphe nuclei of rats. *Neurochem. Res.*, **1997**, *22*(1), 87-92.

[124] Lee, S.H.; Chun, W.; Kong, P.J.; Han, J.A.; Cho, B.P.; Kwon, O.Y.; Lee, H.J.; Kim, S.S. Sustained activation of Akt by melatonin contributes to the protection against kainic acid-induced neuronal death in hippocampus. *J. Pineal Res.*, **2006**, *40*(1), 79-85.

[125] Arisawa, S.; Ishida, K.; Kameyama, N.; Ueyama, J.; Hattori, A.; Tatsumi, Y.; Hayashi, H.; Yano, M.; Hayashi, K.; Katano, Y.;

Goto, H.; Takagi, K.; Wakusawa, S. Ursodeoxycholic acid induces glutathione synthesis through activation of PI3K/Akt pathway in HepG2 cells. *Biochem. Pharmacol.*, **2009**, *77*(5), 858-866.

[126] Kim, S.K.; Woodcroft, K.J.; Khodadadeh, S.S.; Novak, R.F. Insulin signaling regulates gamma-glutamylcysteine ligase catalytic subunit expression in primary cultured rat hepatocytes. *J. Pharmacol. Exp. Ther.*, **2004**, *311*(1), 99-108.

[127] Okouchi, M.; Okayama, N.; Alexander, J.S.; Aw, T.Y. NRF2-dependent glutamate-L-cysteine ligase catalytic subunit expression mediates insulin protection against hyperglycemia- induced brain endothelial cell apoptosis. *Curr. Neurovasc. Res.*, **2006**, *3*(4), 249-261.

[128] Wang, L.; Chen, Y.; Sternberg, P.; Cai, J. Essential roles of the PI3 kinase/Akt pathway in regulating Nrf2-dependent antioxidant functions in the RPE. *Invest. Ophthalmol. Vis. Sci.*, **2008**, *49*(4), 1671-1678.

[129] Halliwell, B. Gutteridge, John M.C. *Free Radicals in Biology and Medicine*. 4th ed. Oxford University Press: Oxford, **2007**, p. 154.

[130] Ma, X.; Idle, J.R.; Krausz, K.W.; Gonzalez, F.J. Metabolism of melatonin by human cytochromes p450. *Drug Metab. Dispos.*, **2005**, *33*(4), 489-494.

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[131] Murawska-Cialowicz, E.; Jethon, Z.; Magdalan, J.; Januszewska, L.; Podhorska-Okolow, M.; Zawadzki, M.; Sozanski, T.; Dziegiel, P. Effects of melatonin on lipid peroxidation and antioxidative enzyme activities in the liver, kidneys and brain of rats administered with benzo(a)pyrene. *Exp. Toxicol. Pathol.*, **2009**, doi:10.1016/j.etp.2009.10.002.

[132] Gomez, M.; Esparza, J.L.; Nogues, M.R.; Giralt, M.; Cabre, M.; Domingo, J.L. Pro-oxidant activity of aluminum in the rat hippocampus: gene expression of antioxidant enzymes after melatonin administration. *Free Radic. Biol. Med.*, **2005**, *38*(1), 104-111.

[133] Reiter, R.J.; Korkmaz, A.; Paredes, S.D.; Manchester, L.C.; Tan, D.X. Melatonin reduces oxidative/nitrosative stress due to drugs, toxins, metals, and herbicides. *Neuro. Endocrinol. Lett.*, **2008**, *29*(5), 609-613.

[134] Cao, X.J.; Wang, M.; Chen, W.H.; Zhu, D.M.; She, J.Q.; Ruan, D.Y. Effects of chronic administration of melatonin on spatial learning ability and long-term potentiation in lead-exposed and control rats. *Biomed. Environ. Sci.*, **2009**, *22*(1), 70-75.

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