

MINIREVIEW

Regulation of antioxidant enzymes: a significant role for melatonin

Abstract: Antioxidant enzymes form the first line of defense against free radicals in organisms. Their regulation depends mainly on the oxidant status of the cell, given that oxidants are their principal modulators. However, other factors have been reported to increase antioxidant enzyme activity and/or gene expression. During the last decade, the antioxidant melatonin has been shown to possess genomic actions, regulating the expression of several genes. Melatonin also influences both antioxidant enzyme activity and cellular mRNA levels for these enzymes. In the present report, we review the studies which document the influence of melatonin on the activity and expression of the antioxidative enzymes glutathione peroxidase, superoxide dismutases and catalase both under physiological and under conditions of elevated oxidative stress. We also analyze the possible mechanisms by which melatonin regulates these enzymes.

**Carmen Rodríguez^{1,2},
Juan C. Mayo^{1,3}, Rosa M. Sainz^{2,3},
Isaac Antolín¹, Federico Herrera¹,
Vanessa Martín¹
and Russel J. Reiter³**

¹Departamento de Morfología y Biología Celular and ²Instituto Universitario de Oncología del Principado de Asturias (IUOPA) Facultad de Medicina, C/ Julian Claveria, Oviedo, Spain; ³Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA

Key words: antioxidant enzyme activity, antioxidant enzyme gene expression, antioxidant enzymes, melatonin, regulation

Address reprint requests to Carmen Rodríguez, Departamento de Morfología y Biología Celular, Facultad de Medicina, c/Julian Claveria, 33006 Oviedo, Spain.
E-mail: carro@correo.uniovi.es

Received July 28, 2003;
accepted September 4, 2003.

Introduction

Aerobic organisms require ground state oxygen to live. However, the use of oxygen during normal metabolism produces reactive oxygen species (ROS), some of which are highly toxic and deleterious to cells and tissues. The most abundant ROS formed in the course of cellular metabolism is the superoxide radical ($O_2^{\cdot-}$). This radical is mainly produced during electron transport in the mitochondria and in the endoplasmic reticulum, although it is also a byproduct in several enzymatic reactions (oxidases and oxygenases); likewise, it is formed during the hepatic metabolism of some molecules and also as a result of the decomposition of oxyhemoglobin [1].

Dismutation of the $O_2^{\cdot-}$ gives rise to hydrogen peroxide (H_2O_2). This molecule is not a free radical per se but, in the presence of transition metals via the Fenton reaction, it is rapidly converted to the hydroxyl radical ($\cdot OH$). The $\cdot OH$ is widely accepted as being the most damaging ROS produced by cells [2]. Free radicals in general and the $\cdot OH$ in particular react with virtually every molecule in living cells (i.e. lipids, sugars, amino acids, nucleotides) with very high rate constants [3]; the resulting damage ultimately may lead to diseases such as cancer, neurodegeneration and autoimmune conditions [4–6].

To protect cells from the damage caused by free radicals and related reactants, organisms have evolved several defense mechanisms to rapidly and efficiently remove ROS from the intracellular environment. When the equilibrium between free radicals (oxidants) and antioxidant defense systems is imbalanced in favor of oxidants, the condition causes what is known as oxidative stress. The oxidants that are not directly scavenged or otherwise not metabolized attack cellular components producing useless molecular debris and sometimes cell death.

Antioxidant defense systems may be generally classified into indirect enzymatic antioxidant enzymes and into small molecular weight molecules which directly scavenge free radicals and related reactants. The antioxidant enzymes represent a first line of defense against these toxic reactants by metabolizing them to innocuous byproducts.

The first enzymatic reaction in the reduction pathway of oxygen occurs during the dismutation of two molecules of $O_2^{\cdot-}$ when they are converted to hydrogen peroxide (H_2O_2) and diatomic oxygen. The enzyme at this step is one of two isoforms of superoxide dismutase (SOD); CuZnSOD is present in the cytosol while (MnSOD) is located in the mitochondrial matrix. These enzymes possess transition metals (Cu^{2+} or Mn^{3+} , respectively) at their active sites; this allows for the rapid exchange of electrons between the

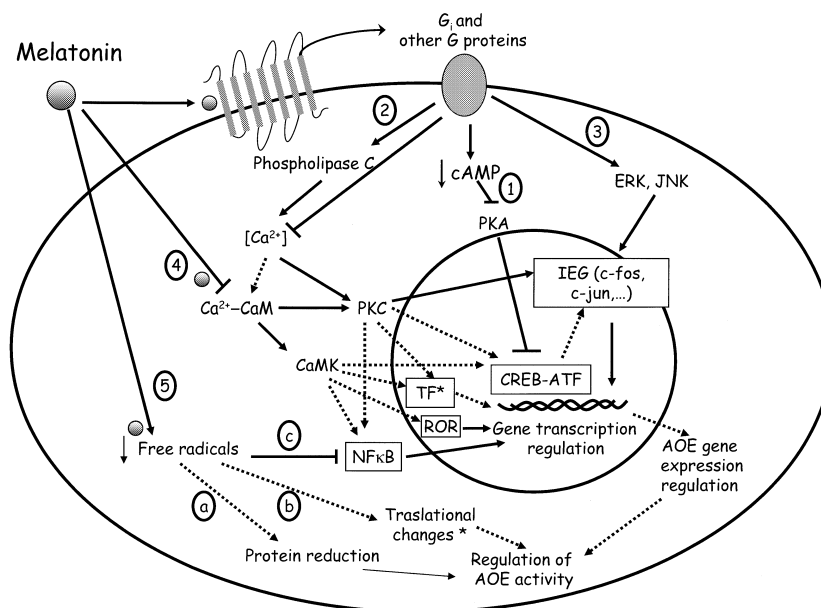


Fig. 1. Hypothetical pathways involved in melatonin regulation of antioxidant enzyme gene expression and activity. (1) Melatonin activation of MT1/2 receptors, via G inhibitory protein (Gi), inhibits adenylyl cyclase and reduces cyclic AMP (cAMP). This results in inhibition of protein kinase A (PKA) and cAMP response element binding protein/activation transcription factor (CREB-ATF). This pathway could modulate immediate early gene (IEG) transcription and consequently gene transcription regulation and antioxidant enzyme concentration. (2) MT1/2 binding by melatonin activates the phospholipase C pathway. The consequent increase in Ca^{2+} concentration will phosphorylate protein-kinase C (PKC) which activates CREB/ATF thereby increasing the transcription of IEG. Indeed, PKC activates IEG. PKC activation may also activate NF kappa B (NFkB) and other transcription factors (TF). Melatonin may also, in other systems, induce a Ca^{2+} decrease leading to inhibition of PKC. (3) MT1/2 activation may, through both inhibitory G (Gi) and other G proteins, activate several mitogen activated protein kinases, i.e., extracellular regulated kinase (ERK) and Jun N-terminal kinase (JNK), which regulate IEG activation and thereby gene transcription. (4) Melatonin may inhibit calcium-calmodulin (Ca-CaM) complex by direct binding. A lowered Ca^{2+} concentration mediated by MT1/2 receptors has been reported in some models. This would inhibit calmodulin-kinase (CaMK), which in turn may regulate NFkB, the retinoid-related receptor (ROR) and other transcription factor activation, thereby influencing gene transcription. Ca^{2+} -CaM inhibition may also regulate PKC. (5) Melatonin is a free radical scavenger. Although this effect is not receptor-mediated, its possible involvement in the regulation of antioxidant enzymes should not be ruled out. Changes in the cellular redox state towards a more reduced environment produces protein reduction which may lead to enzyme activation (a). Also this environment may induce translational changes which would increase enzyme concentrations (b). Finally, a decrease of free radicals would allow repression of redox-sensitive transcription factors (i.e. NFkB, AP-1) which would regulate gene transcription (c). Continuous lines indicate previously reported melatonin actions. Dashed lines indicate general cellular mechanisms previously known but not probed with melatonin. *These effects of melatonin have not been documented.

two superoxides. Although H_2O_2 is not a radical itself, it is reactive and it is rapidly converted into the highly reactive $\cdot\text{OH}$ in the presence of ferrous ion (Fe^{++}) via the Fenton reaction unless it is efficiently removed. Two enzymes participate in the removal of H_2O_2 from the cellular environment, peroxidases and catalase. The most abundant peroxidase is the glutathione peroxidase (GSH-Px), which is present in both the cytosol and mitochondria. This enzyme has the transition metal selenium at its active site and uses reduced glutathione (GSH) as a substrate to transfer electrons to H_2O_2 (and other peroxides) thereby converting it into two molecules of water. The second H_2O_2 metabolizing enzyme is catalase (CAT); it is present mainly in the peroxisomes, presents a molecule of ferric ion at its active site and converts two molecules of H_2O_2 into one molecule each of water and diatomic oxygen [7].

Antioxidant enzymes are regulated by multiple factors. Oxidative status of the cell is the primary factor regulating gene expression and activity of these enzymes [8–10]. Both endogenous [11] and exogenous agents [12, 13] act as oxidants and alter cellular oxidative equilibrium and

therefore antioxidant enzyme gene expression. There are, however, several other factors which influence antioxidant enzymes. In addition to developmental changes, differentiation and aging influences [14–18], inflammation [19, 20] and hormonal regulation of antioxidant enzymes have been reported [21–23]. Additionally, several antioxidants and cell protectors are believed to regulate gene expression and antioxidant enzyme activity [24–29].

Although, melatonin is known to be an indole secreted by the pineal gland, other organs may produce melatonin where it has functions without being released. Besides its properties as a circadian rhythm transducer [30], several other actions for this interesting molecule have been uncovered in the last two decades [31, 32]. Its direct free radical scavenging activity [33, 34] and its regulation of gene transcription [35] for antioxidative enzymes are of special interest in the present review. The antioxidant properties of melatonin have been extensively studied and the use of this molecule as a cell protector and as a potential disease-preventing agent have been summarized [36–40]. Melatonin has been proven to be an efficient oxidant

scavenger of a variety of radical and non-radical reactants [37, 41]. Control of gene expression by melatonin was initially suggested by Menendez-Pelaez et al. [42, 43]. Thereafter, the regulation of expression of several genes related to antioxidative enzymes was reported [24, 44–58]. Herein, the literature related to the regulation of enzyme activity and gene expression of antioxidant enzymes by melatonin is reviewed.

Regulation of antioxidant enzymes by melatonin

Regulation under basal oxidative stress conditions

Reports documenting the influence of melatonin on antioxidant enzyme activity were first published in the mid-1990s [59, 60]. These papers described the amplification of GSH-Px activity in the brain of rat and in several tissues of chicks after exogenously administered melatonin (500 $\mu\text{g/kg}$) [36, 59, 60]. Thereafter, several groups showed that melatonin increases the activity of antioxidant enzymes in other tissues and models. Thus, Ozturk et al. [61] found increased SOD activity in rat liver after administration of 10 mg/kg of melatonin for 7 days, while Liu and Ng [62] reported enhancement of SOD activity in rat kidney, liver and brain after a single melatonin injection (5 mg/kg).

Antioxidant enzyme activities exhibit endogenous rhythms under normal light:dark conditions. This is true both in terms of their activity and gene expression. These changes with time suggested that these cycles might be dependent on the circadian melatonin rhythm [63–65]. Abolition of endogenous melatonin cycle by exposure of animals to constant light, in fact, also abolished the night-time rise in antioxidative enzyme activity. This illustrates that changes in physiological levels of melatonin are adequate to alter the antioxidative defense system as reflected in the level of activities of antioxidative enzymes. Continuous exposure to light is known to abolish the nocturnal melatonin rise; this was associated with a reduction in the night-time increase in GSH-Px and SOD activities in several tissues of chicks [64, 66]. These results were subsequently confirmed by others in rodents [67, 68]. Similarly, Baydas et al. [69] reported that melatonin deficiency caused by pinealectomy reduced GSH-Px activity levels in several tissues of rats.

Melatonin administration during pregnancy has also been shown to stimulate antioxidant enzyme activity in the fetuses. Okatani et al. [70, 71] have reported this finding in both rats [70] and humans [71]. They initially showed that relatively high doses of melatonin (10 mg/kg), administered to pregnant rats, caused incremental changes in the concentration of the indole in both maternal serum and fetal brain as early as 1 hr after its administration. Concomitantly, GSH-Px and SOD activities were likewise increased in fetal brain. This indicates that melatonin may be potentially beneficial in the treatment of stressful conditions that involve free radical production such as fetal hypoxia and preeclampsia. Subsequently, they administered much lower doses of melatonin (100 $\mu\text{g/kg}$ bw) to pregnant women before they underwent voluntary

interruption of pregnancy and they found an increase in GSH-Px activity in chorionic homogenates with a peak 3 hr after indole administration. This again supports the idea that melatonin may have potential usefulness as a fetal protector under conditions of elevated oxidative stress.

Melatonin has also been shown to influence antioxidant enzyme gene expression. As first reported by Antolin et al. [24], melatonin causes incremental changes in mRNA levels for both CuZnSOD and MnSOD in the Harderian gland of female Syrian hamsters after its exogenous administration (500 $\mu\text{g/kg}$). Increases in antioxidant enzyme gene expression following melatonin injections (50 and 500 $\mu\text{g/kg}$) were later confirmed by the same group [52] in rat brain cortex. Finally, Mayo et al. [72] showed that mRNA levels for antioxidant enzymes were elevated in non-differentiated PC12 cells and the human neuroblastoma cells SK-N-SH after melatonin was added to the medium in which the cells were grown. These workers reported that the increases in CuZnSOD and gene HnSOD expression were maximal at 24 and 6 hr, respectively, following melatonin administration. This effect was induced with a melatonin concentration of 10^{-9}M , the physiological levels of this indole in night-time serum; conversely, no effect was observed when higher doses of the indole were used. Regulation of antioxidant enzyme gene expression by melatonin is dependent on new protein synthesis, as use of an inhibitor of protein synthesis, i.e., cycloheximide, prevents mRNA increases after melatonin administration. The indole also reduced the half life of CuZnSOD and GSH-Px while it did not affect that of MnSOD indicating that a larger amount of less stable mRNA may be generated for GSH-Px and CuZnSOD. Finally, the presence of melatonin in the culture medium for 1 hr only is sufficient to increase mRNA for antioxidant enzymes 24 hr later, indicating a possible role for melatonin receptors in the regulation of antioxidant enzymes by this indole.

Regulation under elevated oxidative stress conditions

When cells are exposed to oxidative stress they increase the activity and expression of antioxidant enzymes as a compensatory mechanism to better protect them from the damage induced by free radicals. In many cases the number of free radicals generated may be so great that even the increased activity of the antioxidative enzymes are insufficient to counteract the potential damage. When antioxidant enzyme activities and/or gene expression were examined under highly elevated oxidative stress conditions, it was found that they are sometimes diminished; thus, it has been proposed that moderate levels of toxic reactants induce rises in antioxidant enzymes while very high levels of reactants reduce enzyme activities as a result of damage of the molecular machinery that is required to induce these enzymes [18, 73]. Melatonin has a lengthy history of beneficial actions. For example, almost two decades ago it was reported as a protector against glucocorticoid damage [74, 75], against some degenerative neurological conditions [76], as an anticancer agent [31, 77–79], and also as an enhancer of immune function [32, 79]. Subsequently, the multiple antioxidant properties of melatonin were described

[33, 34, 80, 81] and research on its protective effects against oxidative processes have now been identified under a very wide range of conditions in both experimental animals [82–84] and humans [85, 86]. Some of the earliest studies documented the antioxidant properties of melatonin in the central nervous system [87], in the prevention of cataract formation [88], and in the reduction in the severity of colitis [89]. At roughly the same time, Pablos et al. [60] described the regulation of antioxidant enzyme activities by melatonin; this was quickly followed by studies confirming the original findings and extending the observations of the influence of melatonin on gene expression for antioxidant enzymes.

Antioxidant enzyme regulation by melatonin has been shown to occur concomitant with its protection against elevated oxidative stress in numerous experimental situations. In the first report to document this correlation it was shown that melatonin increased GSH-Px activity and simultaneously reduced free radical damage to the brain and liver of rats treated with lipopolysaccharide (LPS) [90]. In this study, LPS increased total glutathione (tGSH) levels as well as oxidized glutathione (GSSG) concentrations while reducing the activity of GSH-Px. Melatonin (4 mg/kg) given to LPS-treated rats enhanced tGSH above basal levels and lowered GSSG concentrations while stimulating the activity of GSH-Px. This indicated that melatonin may act on several points in the antioxidant defense system, not exclusively on GSH-Px. Subsequently, Antolin et al. [24] reported rises in both CuZn and MnSOD gene expression in the Harderian gland after melatonin (500 µg/kg) was administered to female hamsters. The female hamster Harderian gland is in continual jeopardy of experiencing oxidative stress which causes cell damage because of the extremely high content of porphyrins in this organ. The administration of melatonin lowered porphyrin synthesis and cell damage in this extraorbital tissue and increased gene expression for both isoforms of SOD. In a number of subsequent studies, the activities of both GSH-Px and the SOD were repeatedly shown to be regulated by melatonin with these changes being concurrent with the ability of the indole to reduce oxidative damage.

Multiple reports on neural protection by melatonin via its antioxidant properties have appeared subsequent to the initial reports of this action [81, 90, 91]. In several experiments, antioxidant enzyme activity as well as expression was studied. Mayo et al. [25] found that in an experimental model of Parkinson disease in which dopaminergic PC12 cells were treated with the neurotoxin 6-hydroxydopamine (6-OHDA), low doses of melatonin (10^{-7} M) provided protection against apoptotic death induced by the neurotoxin. In this study, melatonin also prevented the reduction in gene expression for three antioxidant enzymes, GSH-Px, CuZnSOD and MnSOD, which followed 6-OHDA treatments. In vivo experiments have provided results consistent with the in vitro findings. When rodents (rats and mice) were treated with either beta-amyloid peptide 25–35 [92] or with D-galactose [93] both of which cause oxidative damage to the brain, melatonin at doses ranging from 0.1 to 10 mg/kg restored both SOD and GSH-Px activities. Naidu et al. [94] reported reversal of

haloperidol-induced decreases in brain SOD and catalase activities by 1–5 mg/kg melatonin. Melatonin (10 mg/kg or 2 µg/mL in drinking water, respectively) also has been shown to be protective against oxidative stress in both fetal [95] and aging brain of rodents [96], with these beneficial effects being associated with increased GSH-Px activity.

In addition to the brain, antioxidant enzyme activity regulation by melatonin has been shown to be involved in the protection against oxidative damage in other tissues. Restoration or even augmentation of antioxidant enzyme activity by melatonin has been shown to be associated with prevention of free radical damage induced by several toxins [97–99]. For example, intestinal and gastric damage following ischemia-reperfusion or drug administration [100–103], multiple organ damage resulting from therapeutic and non-therapeutic chemotherapeutic agents [104–110], ultraviolet damage to tissues [111], free radical damage in experimental diabetes [112, 113], as well as chemio- and radiotherapy lesions [114, 115] are reduced by melatonin. Finally, it has been recently shown that melatonin may retard aging of the senescence-accelerated mouse with this being associated with augmented antioxidant enzyme activity [96].

Intracellular pathways involved in antioxidant enzyme regulation by melatonin

Mayo et al. [72] provided an insight into the mechanisms by which melatonin regulates antioxidant enzyme gene expression using cultured dopaminergic cells. They found that melatonin induced synthesis of new protein as a condition for regulation of gene expression of all the three antioxidant enzymes, CuZnSOD, MnSOD and GSH-Px. Melatonin also diminished the half-life of mRNAs coding for both CuZnSOD and GSH-Px, without altering that of MnSOD in this study. This indicates that, in the case of the two former enzymes, melatonin in the medium probably induced more abundant levels of mRNAs with shorter half-lives. Finally, nanomolar concentrations of melatonin were adequate to induce antioxidant gene expression with a 1-hr exposure to melatonin being adequate to sustain elevated mRNA levels 24 hr later. As noted above, this points to the likelihood of receptors being involved in antioxidant enzyme gene expression.

The mechanisms involved in the regulation of antioxidant enzymes by melatonin in vivo have not been precisely determined. It is known, however, that stimulation of antioxidant enzyme gene expression occurs at nanomolar concentrations of melatonin in cultured cells [72]; these melatonin levels are equivalent to the serum concentration of melatonin at its nocturnal peak in vivo. The quantities of melatonin used in most of the in vivo experiments, however, very likely caused circulating levels to exceed physiological concentrations. Thus, melatonin in these studies may have functioned as a direct radical scavenger thereby changing the redox state of cells, which in turn may have altered the specific activity of these enzymes or their level of translation [116]. Only twice, as far as could be determined, has gene expression for antioxidative enzymes under the influence of melatonin been analyzed in in vivo experiments [24, 52] and, surprisingly, changes in enzyme activities after

melatonin treatment has not been examined in cell culture experiments.

Kotler et al. [52] found that after chronic administration of melatonin (50 and 500 $\mu\text{g/kg}$) to rats, the lower dose clearly had a greater stimulatory effect on antioxidant enzyme gene expression than did the 500 $\mu\text{g/kg}$ dose. Antolin et al. [117] reported melatonin protection against *in vivo* neurotoxicity of MPTP using 500 $\mu\text{g/kg}$ melatonin (the presumed equivalent melatonin used to induce nanomolar concentrations in serum may be roughly 25–50 $\mu\text{g/kg}$). The work of Barlow-Walden et al. [59] using 500 $\mu\text{g/kg}$ and Kotler et al. [52] using 50 and 500 $\mu\text{g/kg}$, indicate that antioxidant enzyme activity and expression, respectively, are elevated after the administration of melatonin peripherally.

What intracellular molecular pathways are involved in the regulation of antioxidant enzyme gene expression and/or activity by melatonin is presently unknown (Fig. 1). A membrane G-protein-coupled melatonin receptor MT1 was cloned and characterized by Ebisawa et al. [118]. Subsequently, MT2 and Mel 1c receptors have also been identified, the former mainly differing from MT1 in terms of the tissues in which it is expressed, while Mel 1c is not found in mammals [119]. Melatonin also has been tentatively shown to activate a nuclear orphan receptor belonging to the retinoid Z receptor β and α (RZR β and α) family. Melatonin acts on ROR α receptor repressing the expression of the 5-lipoxygenase gene [35] and inhibiting growth of the breast cancer MCF-7 cells [120]. The results from Mayo et al. [72] suggest that melatonin regulation of antioxidant enzymes is receptor-mediated, thereby most likely implicating the MT1/MT2 receptors via second messengers such as cAMP, phospholipase C or intracellular calcium concentration. In addition, binding of melatonin to membrane receptors could stimulate MAP kinase cascades thereby activating several transcription factors [121]. The possibility exists that RZR/ROR receptors could also mediate melatonin effects on antioxidative enzymes as suggested by the results of Pablos et al. [122]; if so, the pathways involved in their regulation obviously remain unknown. One possibility may relate to MT1/MT2 melatonin binding that, through second messengers and phosphorylation cascades, activates RZR/ROR as reported by Ram et al. [120]. Another possibility by which melatonin may regulate RZR/ROR receptors would be via modulation of the calcium/calmodulin signaling pathway, either by changing intracellular calcium concentrations by binding to MT1/MT2 receptors [123], or by direct binding to calmodulin [124]. The calcium/calmodulin signaling pathway has been reported to regulate transcriptional activity of RZR/ROR receptors via CaM kinases [125].

Antioxidant enzymes are known to be regulated by several factors which induce oxidative stress [12, 13, 19, 126]; these factors presumably activate oxidative stress-sensitive transcription factors. Also, transcriptional activation of antioxidant enzyme genes has been reported after the treatment of cells with protective agents [29] where non-oxidative stress-dependent transcription factors are involved. Melatonin has been shown to regulate the activation or repression of several transcription factors [55, 127–130], all of them present in the promoter region of

the three-antioxidant enzymes reviewed herein. Thus, subsequent experiments should be undertaken in order to shed light on the intracellular pathways and transcription factors involved in the regulation of antioxidant enzyme gene expression and activity by melatonin.

Acknowledgments

This work was supported by the CICYT grant no. SAF00-0010, the FICYT grants FC PB MED 01 12 and FC PC REC 01 11, and ASTURPHARMA SA (CR). JCM and VM were supported by FICYT. FH acknowledges a fellowship of the Institute of Health Carlos III (FIS). RMS was supported by a Fulbright Grant.

References

1. HALLIWELL B. Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med* 1991; **91**:3C14S–3C22S.
2. HALLIWELL B, GUTTERIDGE MC. Biologically relevant metal ion-dependent hydroxyl radical generation. *FEBS Lett* 1992; **307**:108–112.
3. HALLIWELL B, GUTTERIDGE MC. The chemistry of oxygen radicals and other oxygen-derived species. In: *Free Radicals in Biology and Medicine*. Halliwell B, Gutteridge JMC, eds., Clarendon Press, Oxford, 1985; pp. 20–66.
4. HALLIWELL B. Oxidants and human disease: some new concepts. *FASEB J* 1987; **1**:358–364.
5. GUTTERIDGE MC. Hydroxyl radicals, iron, oxidative stress, and neurodegeneration. *Ann N Y Acad Sci* 1994; **51**:288–295.
6. FEIG DI, REID TM, LOEB LA. Reactive oxygen species in tumorigenesis. *Cancer Res* 1994; **54**:1890–1894.
7. MATES JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 2000; **153**:83–104.
8. PAHL HL, BAERERLE PA. Oxygen and the control of gene expression. *BioEssays* 1994; **16**:497–502.
9. WARNER BB, STUART L, GEBB S, WISPE JR. Redox regulation of manganese superoxide dismutase. *Am J Physiol* 1996; **271**:L 150–158.
10. FRANCO AA, ODOM RS, RANDO TA. Regulation of antioxidant enzymes gene expression in response to oxidative stress and during differentiation of mouse skeletal muscle. *Free Rad Biol Med* 1999; **50**:2093–2098.
11. NICOTERA TM, NOTARO J, NOTARO S et al. Elevated superoxide dismutase in Bloom's syndrome: a genetic condition of oxidative stress. *Cancer Res* 1989; **49**:5239–5243.
12. YOO HY, CHANG MS, RHO HM. The activation of the rat copper/zinc superoxide dismutase gene by hydrogen peroxide through the hydrogen peroxide-responsive element and by paraquat and heat shock through the same heat shock element. *J Biol Chem* 1999; **274**:23,887–23,892.
13. KIM HP, ROE JH, CHOCK PB, YIM MB. Transcriptional activation of the human manganese superoxide dismutase gene mediated by tetradecanoylphorbol acetate. *J Biol Chem* 1999; **274**:37,455–37,460.
14. HAYASHIBE H, ASAYAMA K, DOBASHI K, KATO K. Prenatal development of antioxidant enzymes in rat lung, kidney, and heart: marked increase in immunoreactive superoxide dismutases, glutathione peroxidase and catalase in the kidney. *Pediatr Res* 1990; **27**:472–475.

15. BRAVARD A, PETRIDIS F, LUCCIONI C. Modulation of anti-oxidant enzymes p21^{WAF1} and p53 expression during proliferation and differentiation of human melanoma cell lines. *Free Rad Biol Med* 1999; **26**:1027–1033.
16. VANELLA A, VILLA RF, GORINI A et al. Superoxide dismutase and cytochrome oxidase activities in light and heavy synaptic mitochondria from rat cerebral cortex during aging. *J Neurosci Res* 1989; **22**:351–355.
17. KASAPOGLU M, OZBEN T. Alterations of antioxidant enzymes and oxidative stress markers in aging. *Exp Gerontol* 2001; **36**:209–220.
18. WEI YH, LEE HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp Biol Med* 2002; **227**:671–682.
19. JONES PL, PING D, BOSS JM. Tumor necrosis factor alpha and interleukin-1 β regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP- β and NF- κ B. *Mol Cell Biol* 1997; **17**:6970–6981.
20. ROGERS RJ, CHESROWN SE, KUO S et al. Cytokine-inducible enhancer with promoter activity in both the rat and human manganese-superoxide dismutase genes. *Biochem J* 2000; **347**:233–242.
21. DOUGALL WC, NICK HS. Manganese superoxide dismutase: a hepatic acute phase protein regulated by interleukin-6 and glucocorticoids. *Endocrinology* 1991; **129**:2376–2384.
22. SAMPATH D, PEREZ-POLO R. Regulation of antioxidant enzyme expression by NGF. *Neurochem Res* 1997; **22**:351–362.
23. SUGINO N, HIROSAWA-TAKAMORI M, ZHONG L et al. Hormonal regulation of copper-zinc superoxide dismutase and manganese superoxide dismutase messenger ribonucleic acid in the rat corpus luteum: induction by prolactin and placental lactogens. *Biol Rept* 1998; **59**:599–605.
24. ANTOLIN I, RODRIGUEZ C, SAINZ RM et al. Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. *FASEB J* 1996; **10**:882–890.
25. MAYO JC, SAINZ RM, URIÁ H et al. Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. *J Pineal Res* 1998; **24**:179–192.
26. LIU CK, KO YJ, CHIANG MT et al. Effect of dietary vitamin E on antioxidant status and antioxidant enzyme activities in Sprague-Dawley rats. *Nutr Cancer* 1998; **32**:95–100.
27. OZTURK-UREK R, BOZKAYA LA, TARHAN L. The effects of some antioxidant vitamin- and trace element-supplemented diets on activities of SOD, CAT, GSH-Px and LPO levels in chicken tissues. *Cell Biochem Funct* 2001; **19**:125–132.
28. NAGATA H, TAKEKOSHI S, TAKAGI T et al. Antioxidative action of flavonoids, quercetin and catechin, mediated by the activation of glutathione peroxidase. *Tokai J Exp Med* 1999; **24**:1–11.
29. KIM YH, PARK KH, RHO HM. Transcriptional activation of the Cu,Zn-superoxide dismutase gene through the AP2 site by Ginsenoside Rb₂ extracted from a medicinal plant, *Panax ginseng*. *J Biol Chem* 1996; **271**:24,539–24,543.
30. CASSONE VM. Effects of melatonin on vertebrate circadian systems. *Trends Neurosci* 1990; **13**:457–464.
31. BLASK DE, HILL SM, ORSTEAD KM, MASSA JS. Inhibitory effects of the pineal hormone melatonin and underfeeding during the promotional phase of 7,12-dimethylbenzanthracene-(DMBA)-induced mammary tumorigenesis. *J Neural Transm* 1986; **67**:125–138.
32. MAESTRONI GJ, CONTI A, PIERPAOLI W. Role of the pineal gland in immunity: II. Melatonin enhances the antibody response via an opiate mechanism. *Clin Exp Immunol* 1987; **68**:384–391.
33. TAN DX, CHEN DX, POEGGELER B et al. Melatonin: a potent endogenous hydroxyl radical scavenger. *Endocr J* 1993; **1**:57–60.
34. TAN DX, POEGGELER B, REITER RJ et al. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. *Cancer Lett* 1993; **70**:65–71.
35. STEINHILBER D, BRUNGS M, WERZ O et al. The nuclear receptor for melatonin repress 5-lipoxygenase gene expression in human B lymphocytes. *J Biol Chem* 1995; **270**:7037–7040.
36. REITER RJ. Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J* 1995; **9**:526–533.
37. REITER RJ. Oxidative damage in the central nervous system: protection by melatonin. *Prog Neurobiol* 1998; **56**:359–384.
38. REITER RJ, MAESTRONI G. Melatonin in relation to the antioxidative defense and immune systems: possible implications for cell and organ transplantation. *J Mol Med* 1999; **77**:36–39.
39. KARBOWNIK M, REITER RJ. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. *Proc Soc Exp Biol Med* 2000; **225**:9–22.
40. REITER RJ, TAN DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. *Cardiovasc Res* 2003; **58**:10–19.
41. ALLEGRA M, REITER RJ, TAN D-X et al. The chemistry of melatonin's interaction with reactive species. *J Pineal Res* 2003; **34**:1–10.
42. MENENDEZ-PELAEZ A, RODRIGUEZ C, DOMINGUEZ P. 5-Aminolevulinatesynthase mRNA levels in the Harderian glands of Syrian hamsters: correlation with porphyrin concentrations and regulation by androgens and melatonin. *Mol Cell Endocrinol* 1991; **80**:177–182.
43. MENENDEZ-PELAEZ A, POEGGELER B, REITER RJ et al. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. *J Cell Biochem* 1993; **53**:373–382.
44. RODRIGUEZ C, KOTLER M, MENENDEZ-PELAEZ A et al. Circadian rhythm in 5-aminolevulinic synthase mRNA levels in the Harderian gland of the Syrian hamster: involvement of light:dark cycle and pineal function. *Endocrine* 1994; **2**:863–868.
45. MOLIS TM, SPRIGGS LL, HILL SM. Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. *Mol Endocrinol* 1994; **8**:1681–1690.
46. WAJS E, KUTOH E, GUPTA D. Melatonin affects proopiomelanocortin gene expression in the immune organs of the rat. *Eur J Endocrinol* 1995; **133**:754–760.
47. REITER RJ, OH C-S, FUJIMORI O. Melatonin. Its intracellular and genomic actions. *Trends Endocrinol Metab* 1996; **7**:22–27.
48. RODRIGUEZ C, KOTLER M, ANTOLIN I et al. Regulation of the aminolevulinic synthase gene in the Syrian hamster Harderian gland: changes during development and circadian rhythm and role of some hormones. *Micro Res Technique* 1996; **34**:65–70.
49. LI S, GIVALOIS L, PELLETIER G. Effects of aging and melatonin administration on gonadotropin-releasing hormones (GnRH) gene expression in the male and female rat. *Peptides* 1997; **18**:1023–1028.

50. TANG YP, MA YL, CHAO CC et al. Enhanced glial cell line-derived neurotrophic factor mRNA expression upon (-)-deprenyl and melatonin treatments. *J Neurosci Res* 1998; **53**:593–604.
51. SAINZ RM, MAYO JC, KOTLER M et al. Melatonin decreases mRNA for histone H4 in thymus of young rats. *Life Sci* 1998; **63**:1109–1117.
52. KOTLER ML, RODRIGUEZ C, SAINZ RM et al. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J Pineal Res* 1998; **24**:83–89.
53. SAINZ RM, MAYO JC, REITER RJ et al. Melatonin regulates glucocorticoid receptor: an answer to its antiapoptotic action in thymus. *FASEB J* 1999; **13**:1547–1556.
54. CRESPO E, MACIAS M, POZO D et al. Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. *FASEB J* 1999; **13**:1537–1546.
55. WON JS, SONG DK, HUH SO et al. Effect of melatonin on the regulation of proenkephalin and prodynorphin mRNA levels induced by kainic acid in the rat hippocampus. *Hippocampus* 2000; **10**:236–243.
56. ROY D, BELSHAM DD. Melatonin receptor activation regulates GnRH gene expression and secretion in GT1-7 GnRH neurons. Signal transduction mechanisms. *J Biol Chem* 2002; **277**:251–258.
57. SHARMAN KG, SHARMAN EH, YANG E, BONDY SC. Dietary melatonin selectively reverses age-related changes in cortical cytokine mRNA levels, and their responses to an inflammatory stimulus. *Neurobiol Aging* 2002; **23**:633–638.
58. POZO D, GUERRERO JM, CALVO JR. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit LPS-stimulated MIP-1 alpha production and mRNA expression. *Cytokine* 2002; **18**:35–42.
59. BARLOW-WALDEN LR, REITER RJ, ABE M et al. Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int* 1995; **26**:497–502.
60. PABLOS MI, AGAPITO MT, GUTIERREZ R et al. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J Pineal Res* 1995; **19**:111–115.
61. OZTURK G, COSKIN 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**:149–153.
62. LIU F, NG TB. 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**:447–453.
63. DIAZ-MUÑOZ M, HERNANDEZ-MUÑOZ R, SUAREZ J, CHAGOYA DE SANCHEZ V. Day–night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. *Neuroscience* 1985; **16**:859–863.
64. ALBARRAN MT, LOPEZ-BURILLO S, PABLOS MI et al. 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**:227–233.
65. MARTIN V, SAINZ RM, MAYO JC et al. Daily rhythm of gene expression in rat superoxide dismutases. *Endocrine Res* 2003; **29**:83–95.
66. PABLOS MI, REITER RJ, ORTIZ GG et al. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. *Neurochem Int* 1998; **32**:69–75.
67. TUNEZ I, MUÑOZ MC, FEJOO M et al. Melatonin effect on renal oxidative stress under constant light exposure. *Cell Biochem Funct* 2003; **21**:35–40.
68. TOMAS-ZAPICO C, COTO-MONTES A, MARTINEZ-FRAGA J et al. Effects of continuous light exposure on antioxidant enzymes, porphyrin enzymes and cellular damage in the Harderian gland of Syrian hamster. *J Pineal Res* 2003; **34**:60–68.
69. BAYDAS G, GURSU MF, YILMAZ S et al. Daily rhythm of glutathione peroxidase activity, lipid peroxidation and glutathione levels in tissues of pinealectomized rats. *Neurosci Lett* 2002; **323**:195–198.
70. OKATANI Y, WAKATSUKI A, KANEDA C. Melatonin increases activities of glutathione peroxidase and superoxide dismutase in fetal rat brain. *J Pineal Res* 2000; **28**:89–96.
71. OKATANI Y, WAKATSUKI A, SHINOHARA K et al. Melatonin stimulates glutathione peroxidase activity in human chorion. *J Pineal Res* 2001; **30**:199–205.
72. MAYO JC, SAINZ RM, ANTOLIN I et al. Melatonin regulation of antioxidant enzyme gene expression. *Cell Mol Life Sci* 2002; **59**:1706–1713.
73. GECHEV T, GADJEV I, VAN BREUSEGEM F et al. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cell Mol Life Sci* 2002; **59**:708–714.
74. MORI W, AOYAMA H, MORI N. Melatonin protects rats from injurious effects of a glucocorticoid, dexamethasone. *Jpn J Exp Med* 1984; **54**:255–261.
75. AOYAMA H, MORI W, MORI N. Anti-glucocorticoid effects of melatonin in young rats. *Acta Pathol Jpn* 1986; **36**:423–428.
76. SANDYK R, KAY SR. The relationship of pineal calcification and melatonin secretion to the pathophysiology of tardive dyskinesia and Tourette's syndrome. *Int J Neurosci* 1991; **58**:215–247.
77. BURNS JK. Administration of melatonin to non-human primates and to women with breast carcinoma. *J Physiol* 1973; **229**:38P–39P.
78. EL-DOMEIRI AA, Das Gupta TK. Reversal by melatonin of the effect of pinealectomy on tumor growth. *Cancer Res* 1973; **33**:2830–2833.
79. PIERPAOLI W, YI C. The involvement of pineal gland and melatonin in immunity and aging. I. Thymus-mediated, immunoreconstituting and antiviral activity of thyrotropin-releasing hormone. *J Neuroimmunol* 1990; **27**:99–109.
80. REITER RJ. Interactions of the pineal hormone melatonin with oxygen-centered free radicals: a brief review. *Braz J Med Biol Res* 1993; **26**:1141–1155.
81. PIERREFICHE G, TOPALL G, COURBOIN G et al. Antioxidant activity of melatonin in mice. *Res Commun Chem Pathol Pharmacol* 1993; **80**:211–223.
82. JAHOVIC N, CEVIK H, OZER SEHIRILI A, YEGEN BC, SENER G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. *J Pineal Res* 2003; **34**:282–293.
83. JAWOREK J, LEJA-SZPAK A, BONIOR J et al. Protective effects of melatonin and its precursor L-tryptophan on acute pancreatitis induced by caerulein overstimulation or ischemia/reperfusion. *J Pineal Res* 2003; **34**:40–52.
84. REITER RJ, SAINZ RM, LOPEZ-BURILLO S, MAYO JC, MANCHESTER LC, TAN DX. Melatonin ameliorates neurologic damage and neurophysiologic deficits in experimental models of stroke. *Ann NY Acad Sci* 2003; **993**:35–47.

85. GITTO E, KARBOWNIK M, REITER RJ et al. Effects of melatonin treatment in septic newborns. *Pediat Res* 2001; **50**:756–760.
86. GITTO E, REITER RJ, CORDARO SP et al. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. *Am J Perinatol* in press.
87. CAGNOLI CM, ATABAY C, KHARLAMOVA E, MANEY H. Melatonin protects neurons from singlet oxygen-induced apoptosis. *J Pineal Res* 1995; **18**:222–226.
88. ABE M, REITER RJ, ORHII PB et al. Inhibitory effect of melatonin on cataract formation in newborn rats: evidence for an antioxidative role for melatonin. *J Pineal Res* 1994; **17**:94–100.
89. PENTNEY PT, BUBENIK GA. Melatonin reduces the severity of dextran-induced colitis in mice. *J Pineal Res* 1995; **19**:31–39.
90. SEWERYNEK E, ABE M, REITER RJ et al. Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals. *J Cell Biochem* 1995; **58**:436–444.
91. MILLER JW, SELHUB J, JOSEPH JA. Oxidative damage caused by free radicals produced during catecholamine autoxidation: protective effects of O-methylation and melatonin. *Free Rad Biol Med* 1996; **21**:241–249.
92. SHEN YX, XU SY, WEI W et al. The protective effects of melatonin from oxidative damage induced by amyloid beta-peptide 25–35 in middle-aged rats. *J Pineal Res* 2002; **32**:85–89.
93. SHEN YX, XU SY, WEI W et al. Melatonin reduces memory changes and neuronal oxidative damage in mice treated with D-galactose. *J Pineal Res* 2002; **32**:173–178.
94. NAIDU PS, SING A, KAUR P et al. Possible mechanism of action in melatonin attenuation of haloperidol-induced orofacial dyskinesia. *Pharmacol Biochem Behav* 2003; **74**:641–648.
95. WAKATSUKI A, OKATANI Y, SHINOHARA K et al. Melatonin protects fetal rat brain against oxidative mitochondrial damage. *J Pineal Res* 2001; **30**:22–28.
96. OKATANI Y, WAKATSUKI A, REITER RJ, MIYAHARA Y. Melatonin reduces oxidative damage of neural lipids and proteins in senescence-accelerated mouse. *Neurobiol Aging* 2002; **23**:639–644.
97. MEKI AR, HUSSEIN AA. Melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney. *Comp Biochem Physiol C Toxicol Pharmacol* 2001; **130**:305–313.
98. EL-SOKKARY GH, OMAR HM, HASSANEIN AF et al. Melatonin reduces oxidative damage and increases survival of mice infected with *Schistosoma mansoni*. *Free Rad Biol Med* 2002; **32**:319–332.
99. HSU CH, CHI BC, LIU MY et al. Phosphine-induced oxidative damage in rats: role of glutathione. *Toxicology* 2002; **179**:1–8.
100. USTUNDAG B, KAZEZ A, DEMIRBAG M et al. Protective effect of melatonin on antioxidative system in experimental ischemia-reperfusion of rat small intestine. *Cell Physiol Biochem* 2000; **10**:229–236.
101. CABEZA J, MOTILVA V, MARTIN MJ, DE LA LASTRA CA. Mechanisms involved in gastric protection of melatonin against oxidant stress by ischemia-reperfusion in rats. *Life Sci* 2001; **68**:1405–1415.
102. OTHMAN AI, EL-MISSIRY MA, AMER MA. The protective action of melatonin on indomethacin-induced gastric and testicular oxidative stress in rats. *Redox Rep* 2001; **6**:173–177.
103. BANDYOPADHYAY D, BANDYOPADHYAY A, DAS PK, REITER RJ. Melatonin protects against gastric ulceration and increases the efficacy of ranitidine and omeprazole in reducing gastric damage. *J Pineal Res* 2002; **33**:1–7.
104. GULTEKIN F, DELIBAS N, YASAR S, KILINC I. In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorophylls-ethyl in rats. *Arch Toxicol* 2001; **75**:88–96.
105. OZBEK E, TURKOZ Y, SAHNA E et al. Melatonin administration prevents the nephrotoxicity induced by gentamicin. *BJU Int* 2000; **85**:742–746.
106. ONER-IYIDOGAN Y, GURDOL F, ONER P. The effects of acute melatonin and ethanol treatment on antioxidant enzyme activities in rat testes. *Pharmacol Res* 2001; **44**:89–93.
107. EL-MISSIRY MA. Prophylactic effect of melatonin on lead-induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rats. *J Biochem Mol Toxicol* 2000; **14**:57–62.
108. ARSLAN SO, ZERIN M, VURAL H, COSKUN A. The effect of melatonin of bleomycin-induced pulmonary fibrosis in rats. *J Pineal Res* 2002; **32**:21–25.
109. CRUZ A, PADILLO FJ, TUNEZ I et al. Melatonin protects against renal oxidative stress after obstructive jaundice in rats. *Eur J Pharmacol* 2001; **425**:135–139.
110. LANKOFF A, BANASIK A, NOWAK M. Protective effect of melatonin against nodularin-induced oxidative stress. *Arch Toxicol* 2002; **76**:158–165.
111. ANWAR MM, MOUSTAFA MA. The effect of melatonin on eye lens of rats exposed to ultraviolet radiation. *Comp Biochem Physiol C Toxicol Pharmacol* 2001; **129**:57–63.
112. SAILAJA DEVI MM, SURESH Y, DAS UN. Preservation of the antioxidant status in chemically-induced diabetes mellitus by melatonin. *J Pineal Res* 2000; **29**:108–115.
113. VURAL H, SABUNCU T, ARSLAN SO, AKSOY N. Melatonin inhibits lipid peroxidation and stimulates the antioxidant status of diabetic rats. *J Pineal Res* 2001; **31**:193–198.
114. WAHAB MH, AKOUL ES, ABDEL-AZIZ AA. Modulatory effects of melatonin and vitamin E on doxorubicin-induced cardiotoxicity in Ehrlich ascites carcinoma-bearing mice. *Tumori* 2000; **86**:157–162.
115. KAYA H, DELIBAS N, SERTESER M et al. The effect of melatonin on lipid peroxidation during radiotherapy in female rats. *Strahlenther Onkol* 1999; **175**:285–288.
116. CLERCH LB, MASSARO D. Tolerance of rats to hyperoxia. Lung antioxidant enzyme gene expression. *J Clin Invest* 1993; **91**:499–508.
117. ANTOLIN I, MAYO JC, SAINZ RM et al. Protective effect of melatonin in a chronic experimental model of Parkinson's disease. *Brain Res* 2002; **943**:163–173.
118. EBISAWA T, KARNE S, LERNER MR, REPPERT SM. Expression cloning of a high-affinity melatonin receptor from *Xenopus* dermal melanophores. *Proc Natl Acad Sci USA* 1994; **91**:6133–6137.
119. REPPERT SM, WEAVER DR, CASSONE VM et al. Melatonin receptors are for the birds; molecular analysis of two receptor subtypes differentially expressed in chick brain. *Neuron* 1995; **15**:1003–1015.
120. RAM PT, DAI J, YUAN L et al. Involvement of the mt1 melatonin receptor in human breast cancer. *Cancer Lett* 2002; **179**:141–150.
121. CHAN A, LAI F, LO R et al. Melatonin mt1 and MT2 receptors stimulate c-Jun N-terminal kinase via pertussis toxin-sensitive and -insensitive G proteins. *Cell Signal* 2002; **14**:249–257.

122. PABLOS MI, GUERRERO JM, ORTIZ GG, AGAPITO MT, REITER RJ. Both melatonin and putative nuclear melatonin receptor agonist CGP 52608 stimulate glutathione peroxidase and glutathione reductase activities in mouse brain in vivo. *Neuroendocrinol Lett* 1997; **18**:49–58.
123. VANECEK J. Cellular mechanisms of melatonin action. *Physiol Rev* 1998; **78**:687–721.
124. BENITEZ-KING G, HUERTO-DELGADILLO L, ANTON-TAY F. Binding of ^3H -melatonin to calmodulin. *Life Sci* 1993; **53**: 201–207.
125. KANE CD, MEANS AR. Activation of orphan receptor-mediated transcription by Ca^{2+} /calmodulin-dependent protein kinase IV. *EMBO J* 2000; **19**:691–701.
126. TANAKA T, KURABAYASHI M, AIHARA Y et al. Inducible expression of manganese superoxide dismutase by phorbol 12-myristate 13-acetate is mediated by Sp1 in endothelial cells. *Arterioscler Thromb Vasc Biol* 2000; **20**:392–401.
127. MOHAN N, SADEGHI K, REITER RJ, MELTZ ML. The neuro-hormone melatonin inhibits cytokine, mitogen and ionizing radiation induced NF-kappa B. *Biochem Mol Biol Int* 1995; **37**:1063–1070.
128. GILAD E, WONG HR, ZINGARELLI B et al. Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NF κ B activation. *FASEB J* 1998; **12**:685–693.
129. ROSS AW, BARRETT P, MERCER JG, MORGAN PJ. Melatonin suppresses the induction of AP-1 transcription factor components in the pars tuberalis of the pituitary. *Mol Cell Endocrinol* 1996; **123**:71–80.
130. URATA Y, HONMA S, GOTO S et al. Melatonin induces γ -glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Rad Biol Med* 1999; **27**:838–847.