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Research Article

Melatonin regulation of antioxidant enzyme gene expression

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Abstract. Antioxidant enzymes (AOEs) are part of the primary cellular defense against free radicals induced by toxins and/or spontaneously formed in cells. Melatonin (MLT) has received much attention in recent years due to its direct free radical scavenging and antioxidant properties. In the present work we report that MLT, at physiological serum concentrations (≈1 nM), increases the mRNA of both superoxide dismutases (SODs) and glutathione peroxidase (GPx) in two neuronal cell lines. The MLT effect on both SODs and GPx mRNA was mediated by a de novo synthesized protein. MLT alters mRNA stability for Cu-Zn SOD and GPx. Experiments with a short time treatment (pulse action) of MLT suggest that the regulation of AOE gene expression is likely to be receptor mediated, because 1-h treatment with MLT results in the same response as a 24-h treatment.

Key words. Melatonin; gene expression regulation; Cu-ZnSOD; MnSOD; glutathione peroxidase; oxidative stress; neurodegenerative disease.

Introduction

Antioxidant enzymes are part of the cellular defense against oxidative stress. Thus, the primary defense against damage by superoxide anion radical (O_2^-) damage and its reactive progeny is the superoxide dismutase (SOD) family of enzymes (EC 1.15.1.1) [1]. Three forms of this protein are currently known in humans, namely Cu-ZnSOD (SOD1, cytosolic), MnSOD (SOD2, mitochondrial), and an extracellular form of Cu-ZnSOD (EC-SOD). All these enzymes catalyze the same reaction, the dismutation of superoxide radical into hydrogen peroxide and oxygen. Other antioxidant enzymes such as glutathione peroxidase (GPx; EC 1.11.1.9) and catalase (EC 1.11.1.6) efficiently detoxify the hydrogen peroxide into water. Together, the enzymatic systems work coordinately, permitting cells to survive in an environment with oxygen and to clear out reactive oxygen species (ROS) formed by other toxic sources [2]. When ROS are too high they overload the cellular detoxifying mechanisms, and a state called oxidative stress appears [3]. Beside the regulation of antioxidant enzymes (AOEs) by the redox status of the cell, evidence for their hormonal modulation is growing rapidly, and it seems to be important in aging, as there is a parallel decrease in endocrine function with age. Levels of several hormones (e.g., growth hormone and prolactin) correlate well with different AOE activities in different tissues [4]. Study of the hormonal regulation in different cells becomes very important for physiological and therapeutic approaches.

The neurohormone melatonin or N-acetyl-5-methoxytryptamine (MLT) is mainly synthesized in the pineal gland of all mammalian species [5], and in non-mammalian vertebrates, invertebrates, algae, bacteria, and a variety of plants [6]. MLT exhibits a direct radical- scavenging ability at high concentrations that has been shown

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relevant in the prevention of different oxidative-stress-related processes such as aging or neurodegenerative disorders [7, 8]. Moreover, other authors and our group have reported a non-scavenging antioxidant role for this hormone in vivo. Among these effects are the inhibition of the pro-oxidant enzyme nitric oxide synthase (NOS) [9] and an increase in the activity and mRNA levels of various antioxidant enzymes [10, 11]. Finally, our group has reported the role of physiological concentrations of MLT in preventing the decrease in AOE mRNA levels when cultured neuronal cells were under oxidative conditions [12].

The aim of the present work was to delineate the effect of normal serum concentrations of MLT on AOE gene expression in different neuronal cell lines under normal conditions (not under oxidative stress) in order to elucidate the physiological role of this hormone in AOE metabolism. We also addressed the mechanisms involved in this regulation.

Materials and methods

Materials and reagents

All cell culture reagents were purchased from Sigma (Spain) except fetal bovine serum (FBS) and horse serum (HS) which were obtained from Linus (Cultek, Spain) and dextran D-70 that was obtained from Amersham (UK). Culture flasks and dishes were acquired from Falcon (Becton Dickinson Bio Science, France). Rat tail collagen type I was purchased from Roche (Mannheim, Germany). MLT was obtained from Aldrich (Sigma, Spain). All other reagents were purchased from Sigma.

Culture of cell lines

PC12 cells were purchased from the American Type Culture Collection (ATCC cat. no. CRL 1721) and were cultured, as originally described [13], in RPMI 1640 supplemented with 10% heat-inactivated HS and 5% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin-B. Cells were maintained in rat tail-collagen-type-I-coated T75 flasks at 37 °C in an atmosphere containing 5% CO_2. For all the experiments, cells were seeded at a density of 5×10^6 cells in 100-mm collagen-coated dishes. Medium was always changed every other day until the beginning of the experiment. Experiments were performed when cells reached 70% confluence. Neuroblastoma cells (SK-N-SH) were obtained from ATCC (cat. no. HTB-10). They were grown following the manufacturers specifications, in 90% Eagle's minimum essential medium (EMEM) supplemented with 1.0 mM sodium pyruvate, 10% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. Cells were maintained in T-75 flasks in 5% CO_2 at 37 °C and medium was changed every other day. For all experiments, cells were seeded in 60-mm dishes until they reached 70% confluence and then experiments were carried out as described below. Eventually, in the indicated experiments, in order to remove steroids present in culture media, serum was depleted of organic components with charcoal-dextran as described elsewhere [14].

MLT was always prepared as 100 mM stock dissolved in 50% dimethylsulfoxide (DMSO) and added directly to the medium from a freshly prepared ×100 stock in Hank's balanced salt solution (HBSS). Control plates were treated with the amount of DMSO used to dissolve melatonin.

Northern blot analysis

Cells were grown in 100-mm dishes and after the time points indicated in the different experiments (see Results) harvested by brief trypsinization, centrifuged at 1000 g for 5 min and washed twice in ice-cold PBS. Extraction of total RNA was performed as previously described [15]. Samples were electrophoresed in 1% agarose gel and RNA was transferred to Hybon N^+ nylon membranes (Amersham) using a Hybaid Vacu-aid vacuum device (Thermo Hybaid, Ashford, UK). Blots were then hybridized with the following probes: a 0.6-kb *Eco*RI fragment from the pUC13 rat Cu-ZnSOD cDNA clone; a pSP65-RMS 1.4-kb *Eco*RI fragment containing rat Mn-SOD cDNA clone; a 0.6-Kb *Eco*RI fragment from the pBGPx-24LK 440 rat GPx cDNA clone and a 1.5-kb *Eco*RI fragment with 18S ribosomal subunit cDNA cloned into Bluescript SK^- (ATCC). Probes were labeled with ^{32}P-dCTP using the Rediprime random prime labeling system (Amersham). After hybridization at 42 °C overnight, blots were exposed for 24 h to X-omat AR films (Kodak).

Quantification of autoradiographies

All autoradiographs from Northern blots shown were scanned with a Hewlett Packard Scanjet 5p. The scanned images were quantified using Scion Image for windows (NIH Image for PC) program downloaded from the web site http://www.scioncorp.com.

Graphical representation of data

Data of each sample obtained from densitometric analysis were previously standardized with an 18S ribosomal subunit value. Control samples of each enzyme were assigned 100% of gene expression in each experiment and the rest of the samples were relativized versus the control value. Experiments were repeated at least four times and a representative experiment is shown in each case.

Results

Time course of the MLT effect on AOE gene expression

Our previous studies showed that MLT treatment for 24 h prevented the decrease in AOE mRNA levels shown by PC12 cells when treated with neurotoxins. These findings led us to study the possible regulation by MLT of AOE gene expression when the cells are under physiological conditions (i.e., not under induced oxidative stress). We tested the physiological concentration of MLT (1 nM; equivalent to serum levels) in a time-course experiment. Results from a representative experiment are shown in figure 1A. MLT induced an increase in Cu-ZnSOD and Mn-SOD mRNA levels in a time-dependent manner. The physiological dose of MLT induced a maximum peak in the mRNA levels for both enzymes after 24 h in the presence of the hormone, although significantly higher levels than controls were found shortly after 6 h of treatment (fig. 1B).

Dose response study of MLT effect on AOE gene expression

Having demonstrated the time course effect of MLT, we studied the response to different doses of the hormone after 6 h of treatment, as at this time, MLT induced a significant increase in the gene expression of both SODs. As represented in figure 2, physiological (1 nM) or a low pharmacological dose of MLT (100 nM) were able to increase the mRNA coding for both SODs and GPx.

Effect of translation and transcription inhibitors on gene expression of AOEs induced by MLT

We tried to delineate the effect of the physiological dose of MLT on GPx and SOD mRNA synthesis using inhibitors of protein synthesis and DNA transcription.

Cycloheximide (CHX) was used as an inhibitor of protein synthesis. It was added 1 h before 1 nM MLT addition and culture was then continued for a futher 24 h. As shown in figure 3, CHX prevented MLT induction of GPx, Cu-ZnSOD, and MnSOD gene expression.

The use of the transcription inhibitor actinomycin D (ACT D) made it possible to study the hypothetical effect of MLT on mRNA stability. MLT was added to the culture medium (except in control groups) 24 h before adding ACT D. SOD and GPx mRNA levels were then measured at the different times indicated in figure 4, which plots the regression curves. MLT induced an increase in AOE gene expression, while ACT D had a different effect on the three enzymes studied. The rate of decay of Cu-ZnSOD and GPx mRNA from MLT treated cells was higher than that from control cells. This result indicates that the mRNAs for Cu-ZnSOD and GPx (fig. 4A, C) in cells stimulated with MLT have lower stability than the mRNA from control cells. In contrast, MnSOD mRNA levels from both control and MLT-treated cells seemed to have virtually the same half-life (fig. 4B).

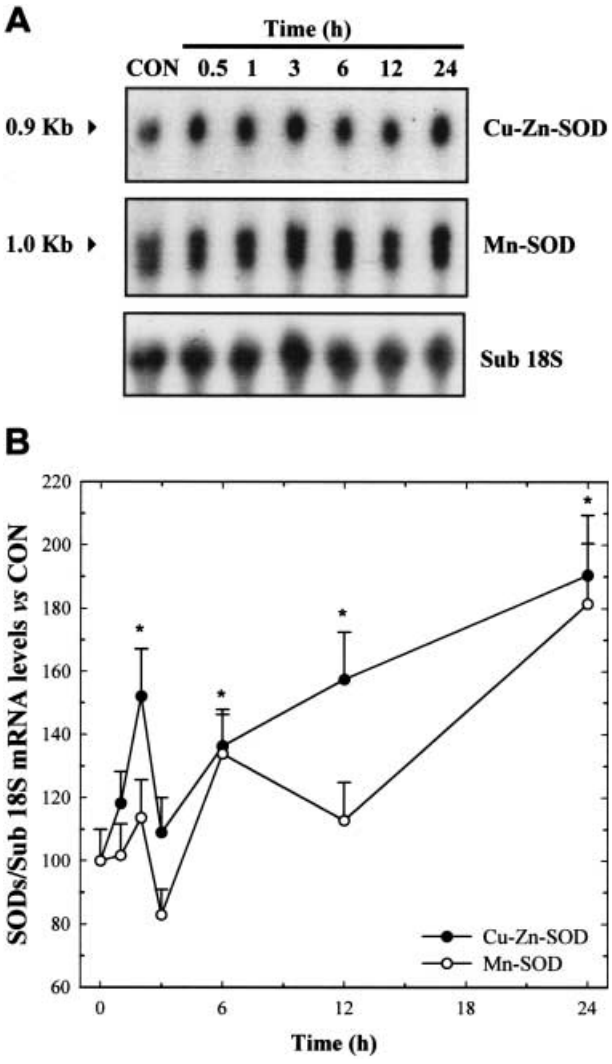

Figure 1. Effect of 1 nM MLT on Cu-ZnSOD and MnSOD mRNA levels. Cells were cultured with 1 nM MLT for the times indicated. (*A*) Northern blot from PC12 cells treated with 1 nM melatonin showing mRNA levels for Cu-ZnSOD, MnSOD and the 18S ribosomal subunit, the latter used as a housekeeping gene. (*B*) Graph obtained from the densitometric analysis of the autoradiographies. Each sample was standardized with the correspondent housekeeping gene signal. Control samples (CON) were considered as 100% gene expression. Results show the mean ± SE from four different experiments. * $p < 0.05$ vs CON.

Effect of short-term treatment with MLT (pulse action) on AOE gene expression

Together, the above results indicated an induction effect likely mediated by a receptor and/or an intermediate protein, because it is restricted to the physiological range of the hormone and prevented by CHX. To examine whether there was a receptor-mediated effect, we performed a set of experiments in which we used a short-term (pulse) treatment with MLT. Cells were treated for 1 h with MLT, and medium was then replaced without the hormone and mRNA levels for AOE were measured after different

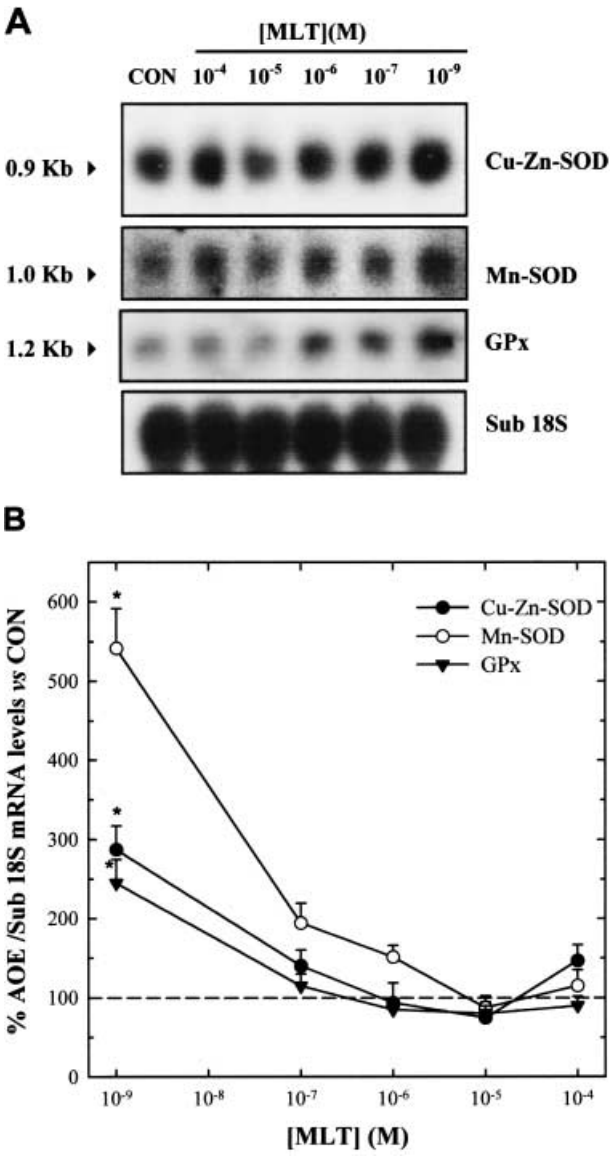

Figure 2. Dose response effect of MLT on Cu-ZnSOD, MnSOD and GPx mRNA levels. Cells were cultured for 6 h with the indicated MLT concentrations. (*A*) Autoradiographies from Northern blots of RNA from PC12 cells showing a dose response study of the MLT effect on Cu-ZnSOD, MnSOD, GPx, and 18S ribosomal subunit mRNA. The latter was used as a housekeeping gene. (*B*) Graph plotting the percentage of gene expression of Cu-ZnSOD, MnSOD and GPx, previously standardized with the housekeeping gene values and related to the control (dashed bar) (see fig. 1 or Materials and methods for details). Results show the mean ± SE from four different experiments. * $p < 0.05$ vs CON.

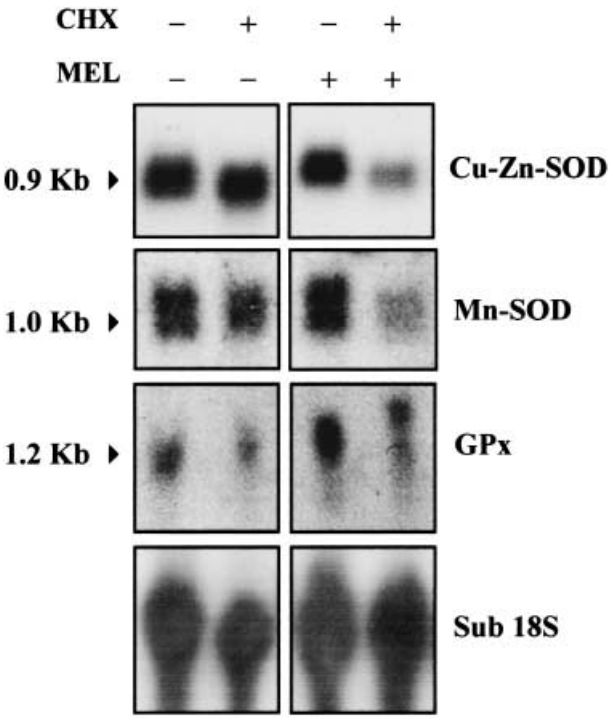

Figure 3. Effect of pre-treatment with the protein synthesis inhibitor cycloheximide (CHX) on MLT stimulation of Cu-ZnSOD, MnSOD and GPx mRNA levels. Cells were pre-treated (or left untreated) for 1 h with CHX and then cultured (or left untreated) with MLT for a further 24 h. Autoradiographies of Northern blots containing RNA from PC12 cells (30 µg) hybridized with rat cDNA of Cu-ZnSOD, MnSOD, GPx, and the 18S ribosomal subunit (housekeeping) are shown. CHX pre-treatment partially prevented the increase in all AOE mRNA levels induced after 24 h of culture in the presence of 1 nM MLT.

times. For this set of experiments, we used medium supplemented with steroid-depleted charcoal-stripped serum to avoid the masking actions due to serum MLT or interactions with steroids and other serum components. Results with PC12 cells are shown in figure 5. AOE mRNA levels reached their maximum level 24 h after the short-term treatment with MLT. One-hour MLT treatment increased AOE gene expression, reaching similar mRNA levels as obtained with the 24-hour treatment, except for GPx. In this case, the mRNA level in the group treated for 24 h with MLT showed a higher induction with respect to the groups incubated for only 1 h with the hormone. Nonetheless, an increase in the groups cultured for 1 h with MLT and then left untreated was also observed compared to control cells. Also observed was a general decrease in AOE mRNA levels in control cells when they were incubated with charcoal-stripped serum, compared to cells cultured with normal serum.

Effect of MLT on AOE mRNA levels in human neuroblastoma cell lines

We extended this MLT study to other cell lines to study whether the MLT effect can be extended to other neuronal cell lines or species. For this purpose, we used neuroblastoma cells. Short-term treatment with MLT induced high AOE mRNA levels in SK-N-SH cells (fig. 6). The timing of the MLT effect differed to that found in PC12 cells. Maximum mRNA levels for all AOEs studied were at 6 h

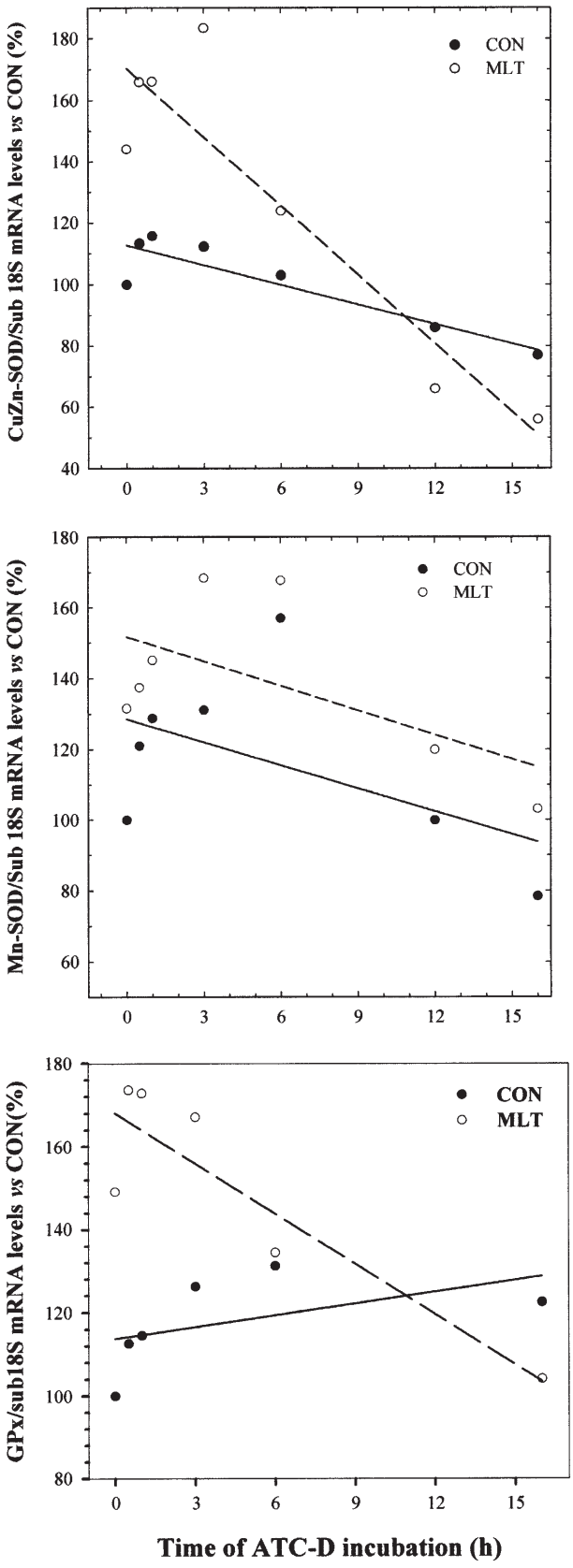

Figure 4. Effect of ACT D in the MLT-induced increase of Cu-Zn-SOD, MnSOD and GPx mRNA levels. Cells were treated with MLT for 24 h or left untreated (CON) and then ACT D was added for the times indicated in both groups. Northern blots containing RNA from PC12 cells (30 μg) were hybridized with rat cDNAs for Cu-ZnSOD, MnSOD, GPx, and the 18S ribosomal subunit (housekeeping). Graph with regression plots of densitometric analysis of mRNA levels for Cu-ZnSOD (*A*), MnSOD (*B*) and GPx (*C*) are shown (——— , CON; ------, MLT).

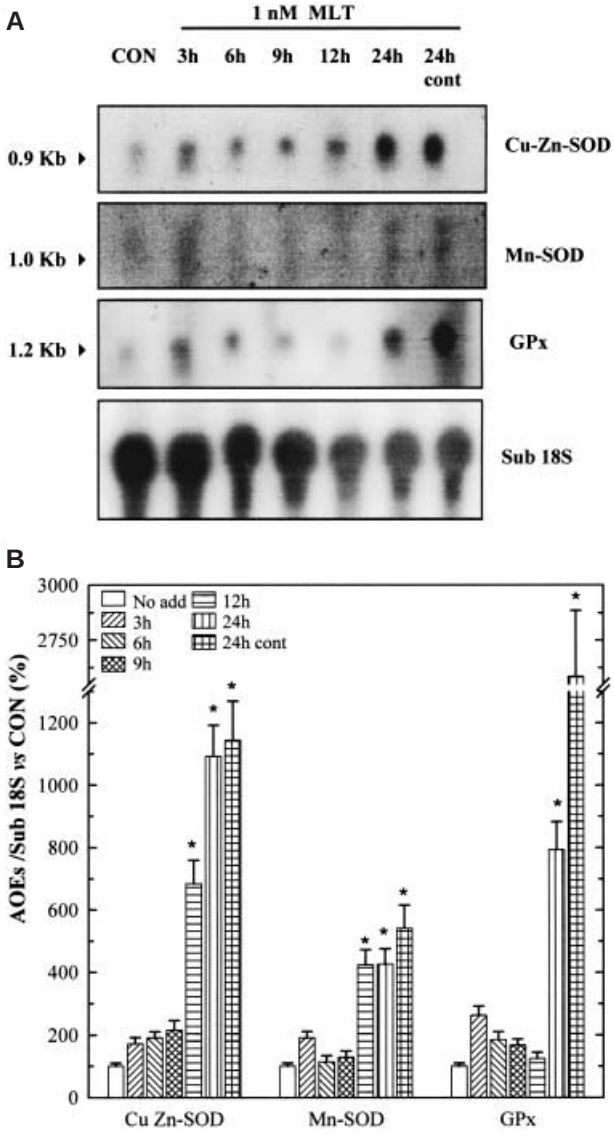

Figure 5. Effect of a short-term treatment with MLT (pulse action) on PC12 cells. Cells were cultured in the presence of 1 nM MLT for 1 h or left untreated (CON) and then changed into medium without MLT. One group was maintained with MLT present in the medium continuously for 24 h. (*A*) Northern blots from PC12 cells showing mRNA levels for Cu-ZnSOD, MnSOD, GPx and the 18S ribosomal subunit (used as housekeeping gene) in a time-course study after the short-term treatment (1 h) with 1 nM MLT. (*B*) Graph representing densitometric analysis of autoradiographies illustrated in *A* with the mRNA levels from control or MLT-treated cells for the times indicated (for details see fig. 1 or Materials and methods). Results show the mean ± SE from four different experiments. * $p < 0.05$ vs CON.

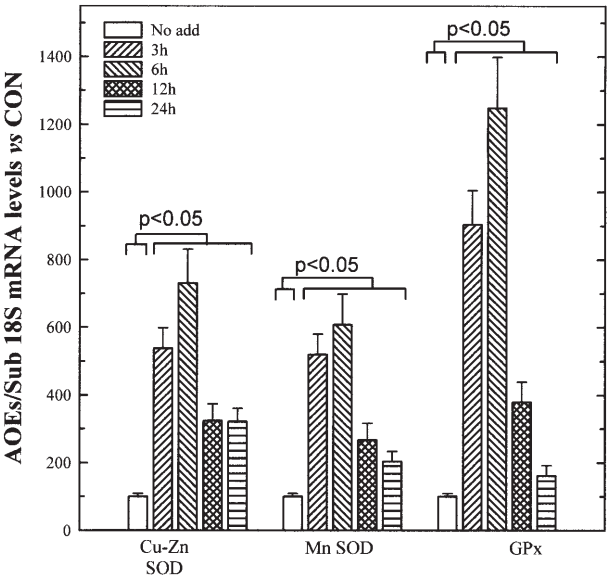

Figure 6. Effect of a short-term treatment with 1 nM MLT on other neuronal cell line (SK-N-SH cells). The graph represents the densitometric analysis of autoradiographies with the mRNA levels for AOE, standardized with housekeeping levels and related to the control (for details see fig. 1 or Materials and methods). Results show the mean ± SE from four different experiments.

and then decreased, although remaining higher than in the controls 24 h after the treatment. As in the case of PC12 cells, 1 h treatment with MLT was enough to induce an increase in the mRNA levels of both SODs and GPx.

Discussion

The data shown in the present paper support a role for MLT at serum concentrations in the physiological regulation of AOEs. Physiological or near-physiological concentrations of MLT stimulate AOE mRNA levels and this regulation seems to be mediated by de novo synthesized protein/s, likely via a receptor. MLT also regulates Cu-ZnSOD and GPx mRNA stability. Together, the results reported here account for a new hormonal regulation of the antioxidant cellular system, in this case by the neurohormone MLT.

Our group previously reported MLT regulation of AOE gene expression in vivo in Harderian gland under porphyrin-induced cell damage [11] and in the brain cortex [16]. We also found prevention by normal serum concentrations of MLT (1 nM) of the decrease in AOE mRNA in PC12 cells treated with the neurotoxin 6-OHDA [12]. In the last model, AOE stimulation is likely to be at least one of the protective mechanisms against 6-OHDA neurotoxicity. To our knowledge, no experiments performed in cell cultures showing the effects of a physiological serum concentration of MLT on AOE gene expression without oxidative stress challenge have been reported until now.

MLT has been studied extensively for its antioxidant properties in several tissues and in different species. It has been proposed as a general protector against neurodegeneration, aging, or against other oxidative-induced diseases [7, 8, 17]. Many of the MLT protection results reported occur in the pharmacological range, MLT acting as a free radical scavenger. However, reports do not exclude, in addition to this effect at high concentrations, a possible action in the serum concentration range.

The results shown in the present work demonstrating MLT-induced gene expression at physiological concentrations and under non-oxidative conditions support the hypothesis that this hormone can work in several ways as an antioxidant, depending on the dose used: inducing gene expression and/or enzyme activity (at low concentrations) or as a direct free radical scavenger (at high concentrations). The previously reported increase in enzyme activity could easily be a consequence of the increased levels of mRNA caused by MLT.

The influence on gene expression described here could be related to some MLT receptor function or other physiological interactions. This can be deduced from experiments in which the culture was treated with MLT for 1 h only and then left up to 24 h without the hormone. These results could be explained in terms of receptor binding or interaction with other intracellular factors. G-coupled membrane receptors of MLT exist, which have been cloned and sequenced [18]. In addition to the membrane-binding sites, nuclear MLT receptors have also been described [19]. Some controversy exists about these, since other authors could not reproduce the binding properties of MLT to RZR/ROR [20]. Nonetheless, RZR/ROR response elements have been found in some important genes, some of them related with oxidative stress. To date, no RZR response elements have been described in the promoters of AOE genes, a point that needs to be considered and investigated more deeply. Although the presence of melatonin receptors in PC12 or SK-N-SH cell lines has not been extensively investigated, the MT1 membrane receptor has been previously demonstrated in PC12 cells [21]. The use of different MLT analogues for membrane or nuclear receptor or membrane protein inhibitors (e.g., pertussis toxin) to mimic or block, respectively, MLT effects on AOEs could provide interesting data about the molecular mechanisms involved. Our results also indicate MLT could regulate different antioxidant enzymes in different ways, because GPx mRNA levels in PC12 cells were higher when MLT was continuously present in the culture medium compared to cells cultured for 1 h and left for 24 h without MLT.

Experiments with CHX imply a role for de-novo-synthesized proteins in the increase of gene expression induced by MLT. These results suggest a possible role for early response gene proteins or other transcription factors. The regulation of γ-glutamylcysteine synthetase (the rate-limit-

ing enzyme in GSH synthesis) gene expression by MLT has been reported recently [22]. The authors show evidence that the AP-1 factor is the mediator of the MLT response. SODs and GPx have response elements for this oxidative stress transcription factor, as well as for NF-κB. Putative response elements for AP-1, Sp1, NF-κB or C/EBP have been found in the 5′-flanking region of the Cu-ZnSOD genomic sequence [23]. NF-κB activation is one of the most important redox-sensitive transcription factors and plays an essential role in gene expression of MnSOD [24]. Since MLT has been reported to regulate NF-κB activation [25], the role of these factors as mediators of MLT action on AOEs should therefore be investigated.

An increase in mRNA levels may be due to an increase in the rate of transcription or an increase in mRNA half-life stability. Results with ACT D demonstrate that there is no effect on MnSOD mRNA stability, suggesting that the increase of this mRNA should be due mainly to an increase in transcription rate. Cu-ZnSOD and GPx mRNA stability in MLT-treated cells was found to be less than in control cells. MLT could increase transcription of these enzymes, with a shorter mRNA half-life. A decrease in SOD mRNA stability also occurs with hypoxia in lung fibroblasts [26] but insights into these mechanisms have not yet been described.

Although hormonal regulation of AOE gene expression has received less attention in the literature than other regulating factors, now there is an increasing interest in the different roles of hormones on the AOE status. Several hormones have been shown to regulate gene expression of AOE [27–30]. Additionally, in MCF-7 cells, a glutathione peroxidase gene, GPx2, is regulated by retinoic acid due to the presence of a retinoic acid response element [31], while both GPx and catalase have been shown to be regulated by NGF [32]. Our results confirm the existence of other hormonal factors that could act as regulators of the AOE gene expression. The importance of knowing all the physiological regulators that can play a fundamental role controlling AOE is essential for the study of the molecular factors that can affect their expression. Additionally, AOE hormonal regulation may account for important therapeutical implications, as the role of these enzymes becomes fundamental in some neurodegenerative disorders or during aging, when hormonal status declines.

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1 Michiels C., Raes M., Toussaint O. and Remacle J. (1994) Importance of Se-glutathione peroxidase, catalase, and Cu-Zn-SOD for cell survival against oxidative stress. Free Radic. Biol. Med. **17:** 235–248
2 Halliwell B. and Cross C. E. (1994) Oxygen-derived species: their relation to human disease and environmental stress. Environ. Health Perspect. **102:** 5–12
3 Halliwell B. and Gutteridge J. M. (1985) Free radicals in Biology and Medicine, Halliwell B. and Gutteridge J. M. C. (eds), Clarendon, Oxford
4 Bolzan A., Brown O., Goya R. and Bianchi M. (1995) Hormonal modulation of antioxidant enzyme activities in young and old rats. Exp. Gerontol. **30:** 169–175
5 Klein D. C., Auerbach D. C., Namboodiri M. A. and Wheler G. H. (1981) Indole metabolism in the mammalian pineal gland. In: The Pinea Gland, vol. I, Anatomy and biochemistry, pp. 199–228, Reiter R. J. (ed.), CRC, Boca Raton
6 Reiter R. J. (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endoc. Rev. **12:** 151–180
7 Reiter R. J. (1995) Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J. **9:** 526–533
8 Giusti P., Lipartiti M., Franceschini D., Schiavo N., Floreani M. and Manev H. (1996) Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. FASEB J. **10:** 891–896
9 Pozo D., Reiter R. J., Calvo J. R. and Guerrero J. M. (1994) Physiological concentrations of melatonin inhibits nitric oxide synthase in rat cerebellum. Life Sci. **55:** 455–460
10 Barlow-Walden L. R., Reiter R. J., Abe M., Pablos M., Menendez-Pelaez A., Chen, L. D. et al. (1995) Melatonin stimulates brain glutathione peroxidase activity. Neurochem. Int. **26:** 497–502
11 Antolin I., Rodriguez C., Sainz R. M., Mayo J. C., Uria H., Kotler M. L. et al. (1996) Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. FASEB J. **10:** 882–890
12 Mayo J. C., Sainz R. M., Uria H., Antolin I., Esteban. M. M. and Rodriguez C. (1998) Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. J. Pineal Res. **24:** 179–192
13 Greene L. A. and Tischler A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA **73:** 2424–2428
14 Osborne C. K., Boldt D. H. and Estrada P. (1984) Human breast cancer cell cycle synchronization by estrogens and antiestrogens in culture. Cancer Res. **44:** 1433–1439
15 Chomczynski P. and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem.**162:** 156–159
16 Kotler M., Rodriguez C., Sainz R. M., Antolin I. and Menendez-Pelaez A. (1998) Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J. Pineal Res. **24:** 83–89
17 Giusti P., Lipartiti M., Gusella M., Floreani M. and Manev H. (1997) In vitro and in vivo protective effects of melatonin against glutamate oxidative stress and neurotoxicity. Ann. N. Y. Acad. Sci. **825:** 79–84
18 Reppert S. M., Weaver D. R. and Ebisawa T. (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron **13:** 1177–1185
19 Wiesenberg I., Missbach M., Kahlen J. P., Schrader M. and Carlberg C. (1995) Transcriptional activation of the nuclear receptor RZR alpha by the pineal gland hormone melatonin and identification of CGP 52608 as a synthetic ligand. Nucleic Acids Res. **23:** 327–333
20 Greiner E., Kirfel J., Greschik H., Dörflinger U., Becker P., Mercep A. et al. (1996) Functional analysis of retinoid Z receptor β, a brain-specific nuclear orphan receptor. Proc. Natl. Acad. Sci. USA **93:** 10105–10110

21 Roth J A., Rosenblatt T., Lis A., and Bucelli R (2001) Melatonin-induced suppression of PC12 cell growth is mediated by its Gi coupled transmembrane receptors. Brain Res. **919:** 139–146.
22 Urata Y., Honma S., Goto S., Todoroki S., Iida T., Cho S. et al. (1999) Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. Free Radic. Biol. Med. **27:** 838-847.
23 Kim H. T., Kim Y. H., Nam J. W., Lee H. J., Rho H. M. and Jung G. (1994) Study of 5′-flanking region of human Cu-Zn superoxide dismutase. Biochem. Biophys. Res. Commun. **201:** 1526–1533
24 White J. E., Lin H. Y., Davis F. B., Davis P. J. and Tsan M. F. (2000) Differential induction of tumor necrosis factor alpha and manganese superoxide dismutase by endotoxin in human monocytes: role of protein tyrosine kinase, mitogen-activated protein kinase, and nuclear factor kappa B. J. Cell. Physiol.**182:** 381–389
25 Lezoualc'h F., Sparapani M. and Behl C. (1998) N-acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF-kappaB. J. Pineal Res. **24:** 168–178
26 Jackson R. M., Parish G. and Ho Y. S. (1996) Effects of hypoxia on expression of superoxide dismutases in cultured ATII cells and lung fibroblasts. Am. J. Physiol. **271:** L955–962
27 Whiteside C., Blackmon R. H. and Bremmer T. A. (1983) Estrogen regulation of superoxide dismutase in normal rat mammary tissues and mammary tumors. Biochem. Biophys. Res. Commun. **113:** 883–887
28 Sugino N., Takiguchi S., Kashida S., Karube A., Nakamura Y. and Kato H. (2000) Superoxide dismutase expression in the human corpus luteum during the menstrual cycle and in early pregnancy. Mol. Hum. Reprod. **6:** 19–25
29 Ramadurai S. M., Nielsen H. C., Chen Y., Hatzis D. and Sosenko I. R. (1998) Differential effects in vivo of thyroid hormone on the expression of surfactant phospholipid, surfactant protein mRNA and antioxidant enzyme mRNA in fetal rat lung. Exp. Lung Res. **24:** 641–657
30 Lareyre J. J., Claessens F., Rombauts W., Dufaure J. P. and Drevet J. R. (1997) Characterization of an androgen response element within the promoter of the epididymi-specific murine glutathione peroxidase 5 gene. Mol. Cell. Endocrinol. **129:** 33–46
31 Chu F. F., Esworthy R. S., Lee L. and Wilczynski S. (1999) Retinoic acid induces Gpx2 gene expression in MCF-7 human breast cancer cells. J. Nutr. **129:** 1846–1854
32 Sampath D. and Perez-Polo R. (1997) Regulation of antioxidant enzyme expression by NGF. Neurochem. Res. **22:** 351–362