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Both Melatonin and a Putative Nuclear Melatonin Receptor Agonist CGP 52608 Stimulate Glutathione Peroxidase and Glutathione Reductase Activities in Mouse Brain In Vivo

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Abstract: Melatonin is both a direct and indirect antioxidant. Thus, it scavenges a variety of free radicals and it stimulates the antioxidative enzyme glutathione peroxidase. Melatonin has been shown to enter the brain and to accumulate in the nucleus of cells. A high-affinity nuclear binding site/receptor for melatonin has been tentatively identified. Using an agonist of the putative nuclear receptor, we show here that the agonist duplicates the stimulatory effect of melatonin on cerebral and cerebellar glutathione peroxidase activity in vivo. We also report that both melatonin and the agonist stimulate glutathione reductase activity. The increases in both enzyme activities are time-dependent, but the stimulation in glutathione reductase activity is delayed compared to that of glutathione peroxidase. The results indicate that melatonin's ability to protect the brain from oxidative damage may be in part be a consequence of a receptor-mediated stimulation of neural antioxidative enzymes.

Zusammenfassung: *Melatonin und das am mutmaßlichen Kern-Rezeptor von Melatonin gleichwirkende CGB 52608 aktivieren die Glutathion-Peroxidase und die Glutathion-Reduktase im Gehirn der Maus in vivo.* Melatonin ist sowohl ein direktes wie auch ein indirektes Antioxidanz. In diesem Sinne tritt es in Kontakt zu einer Vielzahl von freien Radikalen und aktiviert das antioxidative Enzym Glutathion-Peroxidase. Es ließ sich zeigen, daß Melatonin in das Gehirn eindringt und sich in den Zellkernen anreichert. Ein Rezeptor im Zellkern mit hoher Affinität zu Melatonin konnte mit großer Wahrscheinlichkeit identifiziert werden. Bei der Verwendung eines an diesem mutmaßlichen Rezeptors im Zellkern konnten wir zeigen, daß auch der gleichsinnig wirkende Stoff die aktivierende Wirkung von Melatonin auf die Glutathion-Peroxidase im Großhirn und Kleinhirn hat. Wir berichten

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ebenso, daß Melatonin und der genannte gleichsinnig wirkende Stoff die Glutathion-Reduktase aktivieren. Das Anwachsen beider Enzymaktivitäten ist zeitabhängig, aber die Aktivierung der Glutathion-Reduktase ist im Vergleich zu der von Glutathion-Peroxidase verzögert. Die Ergebnisse belegen, daß die Fähigkeit von Melatonin das Gehirn vor oxidativen Schädigungen zu schützen zum Teil eine Konsequenz der rezeptorvermittelten Aktivierung neuraler antioxidativer Enzyme sein könnte.

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Introduction

Melatonin is the neurohormone synthesized by the pineal gland. It was recently shown to be an antioxidant (Reiter et al., 1994; 1995). Melatonin protects tissues from damage induced by free radical-generating substances such as safrole (Tan et al., 1993) and paraquat (Melchiorri et al., 1994). The toxicity of these pro-oxidant agents is thought to be mediated by the production of oxygen free radicals which induce macromolecular damage (Halliwell et al., 1992) including DNA destruction and peroxidation of membrane lipids. Free radical-induced damage is avoided physiologically by both enzymatic and non-enzymatic defense mechanisms. Melatonin is capable of stimulating the detoxifying enzyme glutathione peroxidase (GSH-Px) activity in rat brain (Barlow-Walden et al., 1995) and in several tissues of chicks (Pablos et al., 1995a; 1995b). The increases in GSH-Px activity following melatonin administration are time-dependent. It appears that the ability of melatonin to promote GSH-Px activity could involve the recently defined high affinity nuclear receptor (Acuña-Castroviejo et al., 1993; 1994; Becker-Andre et al., 1994). At least one of these putative nuclear receptors belongs to the orphan superfamily, and a presumed agonist, CGP 52608, has been recently described (Wiesenberg et al., 1995). To check whether nuclear receptors may be involved in melatonin's action on antioxidative enzymes we compared the ability of melatonin and CGP 52608 to stimulate GSH-Px activity in mouse brain in vivo. In the same tissues we measured glutathione reductase (GSSG-Rd) after either melatonin or agonist treatment.

Materials and Methods

Animals and Treatments

Four-week-old male mice (B57/CL) were acclimated to a 14L:10D light:dark cycle (lights off at 21.00 h) and maintained in a temperature ($22 \pm 2\ ^{\circ}C$) and humidity ($45 \pm 5\%$) controlled room with water and food ad libitum.

Nuclear receptor agonist, CGP 52608, was provided by Ciba-Geigy (Basel, Switzerland) and maintained in a solution of dimethylsulfoxide. Both melatonin and the agonist were diluted in saline (diluent). Doses for drugs were chosen according to previous studies (Pablos et al., 1995a; Wiesenberg et al., 1995)

Mice were injected intraperitoneally with diluent (control group), 100 μg/kg agonist CGP 52608 (agonist group), or 500 μg/kg melatonin (melatonin group) at 11.00 h, 4 hours after light onset. Mice were killed at several times after the

injections (60, 90, 120, or 180 min) and both cerebellar and cerebral cortices were collected and frozen until measurements were performed.

Enzyme Activity Measurements

Glutathione peroxidase (GSH-Px) activity was measured by a coupled reaction with glutathione reductase using cumene hydroperoxide as substrate and measuring the decrease in NADPH absorbance at 340 nm as reported previously (Paglia et al., 1967; Bompart et al., 1990). Glutathione reductase (GSSG-Rd) activity was assayed following the decrease in the NADPH absorbance at 340 nm in the presence of oxidized glutathione (Goldberg and Spooner, 1985; Perez-Campo et al., 1993).

Proteins were measured with a kit supplied by BioRad using albumin as standard.

Results

GSH-Px activity was measured in mouse cerebellum and cerebral cortex and results are shown in Fig. 1A and Fig. 2A, respectively. Both tissues exhibited a stimulation in enzyme activity which was maximal 90 min after injections, with a reduction in enzyme activity decreasing after 180 min. The increases in GSH-Px activity were detected following both melatonin and agonist treatments, but the melatonin-induced increase in GSH-Px activity was significantly higher than that induced by the agonist CGP 52608.

In the same tissues, GSSG-Rd activity was also measured and results are shown in Fig. 1B and 2B, respectively. This detoxifying enzyme also exhibited a time-dependent increase with maximal activities being found 120 min after the injections of either melatonin or CGP 52608. In these cases, enzyme activities had also decreased by 180 min. No significant differences were detected in degree of GSSG-Rd stimulation between melatonin and agonist treatments.

Discussion

Melatonin readily enters cells where highest concentrations appear to accumulate in the nuclei (Menendez-Pelaez et al., 1993; Menendez-Pelaez et al., 1994). Nuclear melatonin has been identified using both immunocytochemistry and radioimmunoassay methods. In the nucleus, melatonin binds to a family of high affinity orphan nuclear receptors including RZRβ, which is activated at low nanomolar melatonin concentrations, and RZRα and RORα1 which are proposed to be involved in melatonin-modulated transcriptional regulation in peripheral tissues (Becker-Andre et al., 1994). Also, an agonist of the nuclear receptor has been identified (Wiesenberg et al., 1995). This agonist, CGP 52608 (1-[3-allyl-4-oxo-thiazolidine-2-ylidiene]-4-methyl-thiosemi-carbazone), was determined to be a specific ligand of the RZR receptor with an EC_{50} three times higher than the EC_{50} for melatonin. Although melatonin has been shown to inhibit 5-lipoxygenase gene expression in B-lymphocytes (Steinhilber et al., 1994; Carlberg and Wiesenberg, 1995), probably via an action of melatonin on nuclear receptors, changes

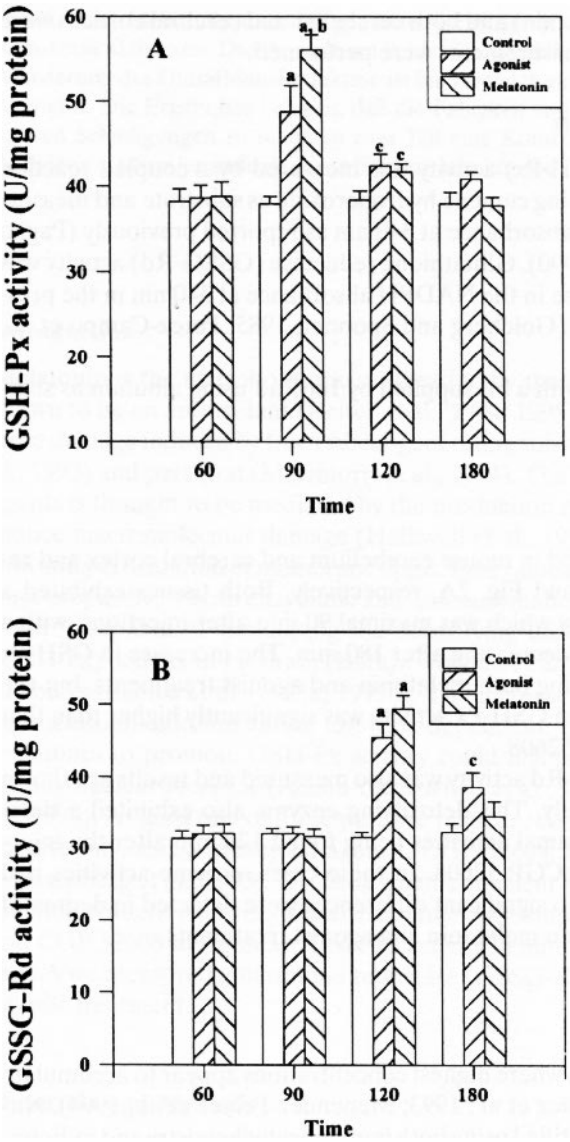

Fig. 1. Increases in GSH-Px activity (A) and GSSG-Rd activity (B) in mouse cerebellum following their treatment with either melatonin or a nuclear melatonin receptor agonist, CGP 52608. [a] $p < 0.005$ verses control group; [b] $p < 0.05$ verses agonist group; [c] $p < 0.05$ verses control group.

that definitively involve melatonin binding to the nuclear receptors have yet to be described.

The issue of whether there is or is not a nuclear binding site for melatonin has been actively debated. Recently, the issue was again discussed in the journal that published one of the original papers showing the binding of melatonin to the RZR/ROR orphan receptor (see Additions and Corrections, 1997). While at this

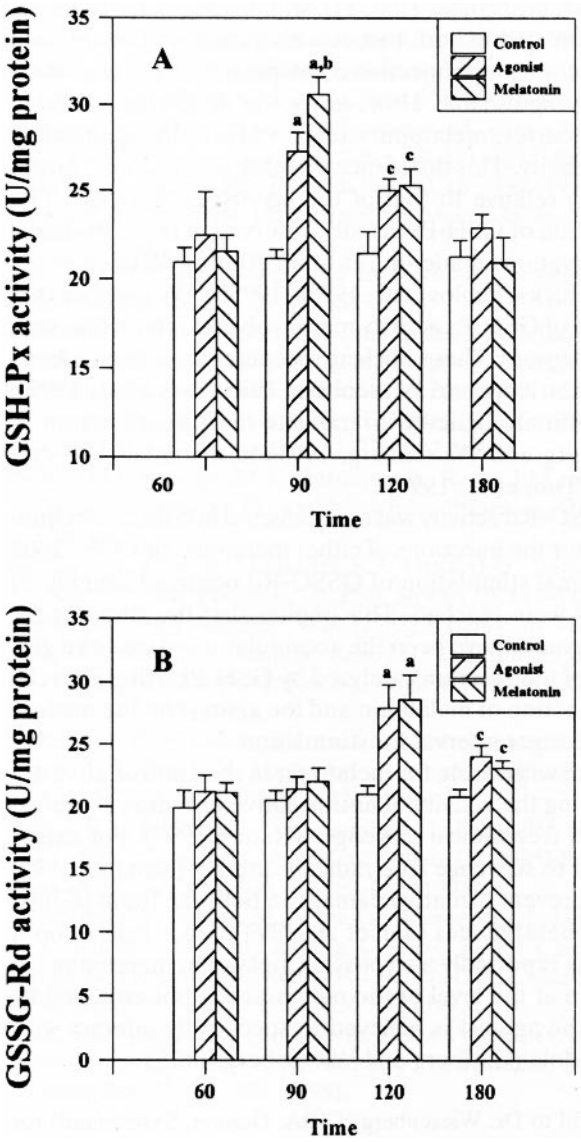

Fig. 2. Increases in GSH-Px activity (A) and GSSG-Rd activity (B) in mouse cerebral cortex following their treatment with either melatonin or a nuclear melatonin receptor agonist, CGP 52608. [a] $p < 0.005$ verses control group; [b] $p < 0.05$ verses agonist group; [c] $p < 0.05$ verses control group.

time there is no known alternative mechanism whereby CGP 52608 could have influenced the activities of the enzymes in question, it is possible that a nuclear binding site for the agonist was not involved.

In the present study, the activities of two detoxifying enzymes, GSH-Px and GSSG-Rd, were measured after either melatonin or nuclear receptor agonist injections into mice. The tissues in which enzyme activities were measured included

the cerebellum (Fig. 1) and cerebral cortex (Fig. 2). An increase in GSH-Px activity (Figs. 1A and 2A) was detected in both tissues with maximal values 90 min after either melatonin or receptor agonist injections. The profiles of the responses to these compounds were indistinguishable. However, at the 90 min time point in both cerebellum and cerebral cortex, melatonin caused statistically significantly higher increases in GSH-Px activity. This difference possibly relates to the larger dose of melatonin (500 μg/kg) relative to that of the agonist (100 μg/kg). The melatonin-dependent stimulation of GSH-Px activity observed in these studies is consistent with previous observations made on rat brain (Barlow-Walden et al., 1995) and in several tissues of chicks (Pablos et al., 1995a; 1995b). We propose that melatonin-induced stimulation of GSH-Px activity may involve its interaction with the RZR-family of nuclear receptors. These nuclear receptors have been identified in the mammalian brain (Carlberg and Wiesenberg, 1995; Park et al., 1997). Nuclear receptors for melatonin are believed to mediate melatonin's action as 5-lipoxygenase activity (Carlberg and Weisenberg, 1995) in mammals and cyst formation in a dinoflagellate (Tsim et al., 1996).

A significant increase in GSSG-Rd activity was also detected in both cerebellum and cerebral cortex 120 min after the injections of either melatonin or CGP 52608 (Figs. 1B and 2B). Thus, maximal stimulation of GSSG-Rd occurred roughly 30 min after peak GSH-Px levels were reached. This implies that the stimulus for the increased GSSG-Rd activity may have been the accumulation of oxidized glutathione which is the product of the reaction catalyzed by GSH-Px. Alternatively, it could also be due to a direct action of melatonin and the agonist on the nuclear receptor but merely require a longer interval for stimulation.

These findings are consistent with a role for melatonin in the antioxidative defense system. Besides, promoting the activity of antioxidative enzymes, as shown here, melatonin is also a direct free radical scavenger (Reiter, 1997). For example, melatonin has been found to scavenge free radicals directly (Scaiano, 1995; Chan and Tang, 1996) and to prevent oxidative damage in both the brain (Giusti et al., 1996) as well as in peripheral tissues (Tan et al., 1993). Since cells in both cerebellar and cerebral cortices reportedly also possess melatonin membrane receptors, an action of melatonin at the level of the membrane is not excluded by these observations, although the agonist is believed to specifically interact with the nuclear receptor for the indole (Carlberg and Wiesenberg, 1995).

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References

Acuña-Castroviejo, D., Pablos, M.I., Menendez-Pelaez, A., and Reiter, R.J. Res. Commun. Chem. Pathol. Pharmacol. 82, 253–256 (1993).

Acuña-Castroviejo, D., Reiter, R.J., Menendez-Pelaez, A., Pablos, M.I., and Burgos A. J. Pineal Res. 16, 100–112 (1994).

Additions and Corrections. J. Biol. Chem. 272, 1607 (1997).

Barlow-Walden, L.R., Reiter, R.J., Abe, M., Pablos, M.I., Menendez-Pelaez, A., Chen, L.D., and Poeggeler, B., Neurochem. Int. 26, 497–502 (1995).
Becker-Andre, M., Wiesenberg, I., Schaeren-Wiemers, N., Andre, E., Missbach, M., Saurat, J.H., and Carlberg, C. J. Biol. Chem. 269, 28531–28534 (1994).
Bompart, B.J., Prevot, D.S., and Bascands, J.L. Clin. Chem. 23, 501–504 (1990). Carlberg, C. and Wiesenberg, I. J. Pineal Res. 18, 171–178 (1995).
Chan, T.Y. and Tang P.L. J. Pineal Res. 20, 187–191 (1996).
Giusti, P., Franceschini, D., Petrone, M., Manev, H. and Floreani, M. (1996) J. Pineal Res. 20, 226–231 (1996).
Goldberg, D.M. and Spooner, R.J. In: Methods in enzymatic analysis. Ed. H.U. Bergmeyer. 3rd ed. Vol. III, 258–265 (1985).
Halliwell, B., Gutteridge, J.M.C., and Cross, C.E. J. Lab. Clin. Med. 119, 598–620 (1992).
Melchiorri, D., Reiter, R.J., Attia, M., Burgos, A., and Nistico, G. Life Sci. 56, 83–89 (1994).
Menendez-Pelaez, A., and Reiter, R.J. J. Pineal Res. 15, 59–67 (1993).
Menendez-Pelaez, A., Poeggeler, B., Reiter, R.J., Barlow-Walden, L.R., Pablos, M.I., and Tan, D.X. J. Cell. Biochem. 53, 373–382 (1994).
Pablos, M.I., Agapito, M.T., Gutierrez, R., Recio, J.M., Reiter, R.J., Barlow-Walden, L.R., Acuña-Castroviejo, D., and Menendez-Pelaez, A. (1995a) J. Pineal Res. 19, 111–113 (1995a).
Pablos, M.I., Chuang, J.I., Reiter, R.J., Ortiz, G.G., Daniels, W.M.U., Sewerynek, E., Melchiorri, D., and Poeggeler, B. Biol. Signals 19, 111–115 (1995b).
Paglia, D.E., and Valentine, W.N. (1967) J. Lab. Clin. Med. 70, 158–169 (1967).
Park, H.T., Kim, Y.J., Yoon, S., Kim, J.B., and Kim, J.J. Brain Res. 747, 332–337 (1997).
Perez-Campo, R., Lopez-Torres, M., Rojas, C., Cadenas, S., and Barja, G. Biochem. Physiol. 105B, 749–755 (1993).
Reiter, R.J. (1997) Antioxidant actions of melatonin. Adv. Pharmacol. 38, 103–117.
Reiter, R.J., Tan, D.X., Poeggeler, B., Menendez-Pelaez, A., Chen, L.D., and Saarela, S. Ann. N.Y. Acad. Sci. 719, 1–12 (1994).
Reiter, R.J., Melchiorri, D., Sewerynek, E., Poeggeler, B., Barlow-Walden, L.R., Chuang, J.I., Ortiz, G.G., and Acuña-Castroviejo, D. (1995) J. Pineal Res. 18, 1–11 (1994).
Scaiano, J.C. J. Pineal Res. 19, 189–195 (1995).
Steinhilber, D., Brungs, M., Werz, O., Wiesenberg, I., Danielsson, C., Kahlen, J.P., Nayeri, S., Schrader, M., and Carlberg, C. (1994) J. Biol. Chem. 270, 7037–7040 (1994).
Tan, D.X., Poeggeler, B., Reiter, R.J., Chen, L.D., Chen, S., Manchester, L.C., and Barlow-Walden, L.R. Cancer Lett. 70, 65–71 (1993).
Tsim, S.T., Wong, J.T.Y., and Wong, T.H. J. Pineal Res. 21, 101–107 (1996).
Wiesenberg, I., Missbach, M., Kahlen, J.P., Schrader, M., and Carlberg, C. (1995) Nucl. Acids Res. 23, 327–333 (1995).