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Antioxidative effect of melatonin in rat brain oxidative stress induced by Adriamycin

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The effect of melatonin administration on the oxidative stress induced by a high dose of Adriamycin (AD, doxorubicin hydrochloride) in plasma, hypothalamus and brain cortex of rats, as well as lipoperoxide changes, and catalase activity (CAT) levels have been studied. After administration of a single high AD dosis (25 mg/kg, i.p.), melatonin was injected daily three days before and after oxidative stress induction. The AD injection produced a significant lipoperoxide increase in plasma, hypothalamus and brain cortex, which was prevented by melatonin. CAT activity mean values decreased in hypothalamus by AD, effect which was reverted and increased by simultaneous melatonin administration. CAT activity was not changed after AD, melatonin or AD + melatonin administration in plasma and brain cortex. These results, especially those concerning lipoperoxide content changes, showed a powerful antioxidative effect of melatonin at both neural and extraneural levels in rats. CAT changes in the presence of melatonin suggest that there is a relationship between a scavenger role of the pineal hormone and a high oxidative activity in the brain hypothalamy area. When these results are taken together, they also show that melatonin, besides, producing the extraneural effect, can act as a powerful antioxidative agent in organs such as the brain, very rich in lipid susceptible to oxidation in the neuronal as well as the extraneuronal tissues.

Key words: Melatonin, Oxidative stress, Rat brain.

Melatonin or N-acetyl-5-methoxytryptamine is the main product of the pineal gland in many vertebrates. FOA (3) established the involvement of the pineal gland, through melatonin action, in the modulation of many different neuroendocrine functions (4, 12), as well as a biological

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clock in response to light and photoperiods to maintain both circadian rhythms (13).

Other melatonin roles have been described, particularly its powerful effect as antioxidant, previously surmised on the basis of two properties of this molecule: 1) its extremely easy diffusivity through biological membranes, due to its lipophilic character, and 2) its lack of specific receptors for melatonin in many tissues (14). More recently, melatonin has been confirmed as a powerful scavenger of OH^-, one of the most reactive and deleterious free radicals produced in the oxidative metabolism (11, 14).

In the present work we show the antioxidant effects of melatonin on the oxidative stress induced by a high single dose of Adriamycin (AD) injected into rats. These results indicate that melatonin was a powerful inhibitor of lipoperoxide formation in rat plasma, hypothalamus and brain cortex

Materials and Methods

Animals.– Male Wistar rats weighing 140-170 g each were obtained from Charles River (Barcelona, Spain). They were housed at 21-23 °C under a light regimen of 14:10 (14 h L/10 h D) with food and water *ad libitum*.

Experimental procedure.– Rats were injected i.p. with AD, melatonin alone and AD+melatonin. AD was administered i.p. as a single dose (25 mg/kg) whereas melatonin (50 µg/animal) alone or associated, was injected i.p. during six days, three before and three after AD administration. Fresh injections were prepared each day by dissolving the powder in 30 % ethanol and normal saline (50 µg/0.1 ml sol./day).

Animals were sacrificed by decapitation three days after administration and treatment with melatonin. Trunk blood was collected to determine lipoperoxide content and catalase activity (CAT) in plasma. Brains were rapidly extracted and homogenates of hypothalamus and brain cortex were prepared and weighed according to described procedures (2, 16). Aliquots from three extracts were used to stimulate protein, lipoperoxides and CAT activity.

Analytical and enzymatic determinations.– Lipoperoxides, referred to as thiobarbituric acid reactive substances (TBARS), were determined spectrophotometrically at 532 nm with thiobarbituric acid (9). Catalase activity was estimated by following the absorbance changes at 240 nm of H_2O_2 disappearance in phosphate buffer, pH 7.0 (1). One unit µmol of H_2O_2 per minute under optimal assay conditions. Protein were determined (5) using bovine serum albumin as a standard.

Chemicals.– Thiobarbituric acid, 1,3,3-tetrametoxypropane, sodium dodecyl sulfate, and melatonin were purchased from Sigma and adriamycin chlorhydrate from Laboratorio Farmitalia S.A.

Statistical analysis of data was performed using the Student *t* test.

Results

Plasma.– Table I shows a significant increase ($p < 0.001$) in plasma TBARS content after AD administration, whereas melatonin prevented it. The antagonistic effect of melatonin is observed since it depressed TBARS content down to levels below those of controls ($p < 0.001$ vs AD and $p < 0.005$ vs control). CAT activity did not change in any condition studies. Besides, melatonin did not produce any change in untreated AD animals (table II).

Hypothalamus.– As in plasma, AD significantly raised TBARS content ($p <$

Table I. *Changes in TBARS in plasma, hypothalamus and brain cortex from rats injected with AD and antioxidative treatment.*
Rats (n=6) were injected with (25 mg/kg), and melatonin (MEL, 50 μg/kg) during 6 days.

	Plasma (nmol / L)	Hypothalamus (nmol / mg protein)	Brain cortex (nmol / mg protein)
Control	3.5 ± 0.3	1.73 ± 0.15	0.30 ± 0.01
MEL	3.9 ± 0.4	1.70 ± 0.19	0.35 ± 0.02
AD	6.4 ± 0.5***	3.00 ± 0.51***	0.41 ± 0.01**
AD + MEL	2.4 ± 0.3•••	2.10 ± 0.25••	0.18 ± 0.005•••

**p<0.01; ***p<0.001 vs control; ••p< 0.01 and •••p< 0.001 vs AD.

Table II. *Changes in CAT activity in plasma, hypothalamus and brain cortex from rats injected with AD and antioxidative treatments.*
Rats (n=6) were injected with AD (25 mg/kg), and MEL (50 μg/kg) during 6 days.

	Plasma (U / mg protein)	Hypothalamus (U / mg protein)	Brain cortex (U / mg protein)
Control	0.07 ± 0.002	0.22 ± 0.02	0.018 ± 0.001
MEL	0.06 ± 0.006	0.19 ± 0.02	0.015 ± 0.001
AD	0.07 ± 0.006	0.16 ± 0.01*	0.015 ± 0.002
AD + MEL	0.08 ± 0.007	0.40 ± 0.03•••	0.015 ± 0.001

*p<0.05; ***p<0.001 vs control and •••p< 0.01 and •••p< 0.001 vs AD.

0.001), this increased being prevented by the simultaneous administration of melatonin (table I). Melatonin injected to control animals, as occurred in plasma, prevented TBARS enhancement. Hypothalamic CAT is decreased by AD (table II). This effect was prevented by melatonin. CAT remained unaltered in animals only injected with melatonin.

Brain cortex.– Changes of TBARS and CAT in brain cortex of treated rats are presented in tables I and II. AD treatment slightly raised TBARS values, which were prevented by melatonin. CAT levels were not significantly altered after the different treatments.

Discussion

The above results indicate that melatonin has a potent antioxidant effect in the three study media, especially in plasma and hypothalamus. These effects on lipoperoxide content are in agreement with similar results found in rats treated with oxidative stress inducers such as alloxan and streptozotocin (10), hydrogen peroxide (15) and kainic acid (6), where melatonin significantly reduced lipoperoxide formation in homogenates from different brain areas. Furthermore melatonin provided a nearly total protection against toxic effects mediated by oxygen free radicals in lung and liver after paraquat administration in rats (8). Paraquat (1-1'-dimethyl-4,4'- bipyridinium) is a highly toxic herbicide which promoted significantly higher levels of 4-hydroxyalkenals and malonaldehyde, which are indicative of lipid peroxidation (7). In our study, the protecting melatonin effect in oxidative stress is confirmed not only by the clear decrease in TBARS, but also by other effects related to the ascitis extent, lethality and other hepatonephrotoxicity signals

produced by the high dose of AD (results not presented).

CAT activity, a peroxisomal enzyme which catalyzes the disproportionation of H_2O_2 into H_2O and O_2, decreased after an AD injection in hypothalamus. Melatonin not only prevented this CAT decrease in hypothalamus but produced a significant enhancement of catalase activity.

Therefore, these results corroborate the fact that melatonin acts as a powerful antioxidant, both in peripheral tissues and in CNS, in the oxidative stress induced by AD in rats. Such an outstanding capacity of melatonin to prevent lipoperoxide formation constitutes an important defense system for the brain, since in this organ there exists a high possibility of production of lipoperoxides due to a high concentration of unsaturated fatty acid and O_2 consumption.

The increase of CAT activity in hypothalamus after treatement with melatonin requires further data for its full explanation. The possibilities of melatonin as antioxidant are unexplored and very promising especially if its main properties are considered, namely its ability to neutralize the most active toxic free radicals known (OH^*), its easy diffusion through membranes, and its lack of requirement for specific receptors to exert its action.

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P. MONTILLA, I. TÚNEZ, M. C. MUÑOZ, J. V. SORIA y A. LÓPEZ. *Efecto antioxidante de la melatonina en el cerebro de rata en el estrés oxidativo inducido por adriamicina*. J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (3), 301-306, 1997.

El efecto de la administración de melatonina sobre el estrés inducido por una alta dosis de adriamicina (AD, doxorrubicina hidroclorada) en plasma, hipotalamo y corteza cerebral de rata, pone de manifiesto cambios en los niveles de lipoperóxidos y actividad catalasa (CAT). Los animales se tratan durante tres días antes y después de la única y alta dosis de AD (25 mg/kg i.p.), con melatonina (50 µg/kg i.p.). La inyección de AD produce incrementos de los lipoperóxidos en plasma, hipotálamo y corteza cerebral. Los valores medios de la actividad CAT descienden en hipotálamo tras AD, efecto que se revierte e incrementa por la simultánea administración de melatonina. Ni AD, ni melatonina sola o AD + melatonina causan cambios significativos de la actividad CAT en plasma y corteza cerebral. Estos resultados, especialmente los referidos a los cambios de los niveles de lipoperóxidos, muestran el poderoso efecto antioxidante de la melatonina, tanto en áreas neurales como extraneurales, en rata. Los cambios de CAT en presencia de melatonina sugieren una relación entre la función antioxidante de la hormona pineal y la alta actividad oxidativa del área hipotalámica cerebral. Estos resultados ponen de manifiesto que la melatonina puede actuar como un potente antioxidante en un órgano como el cerebro, rico en lípidos, compuestos muy propensos a oxidarse en tejidos tanto neuronales como extraneuronales.

Palabras clave: Melatonina, Estrés oxidativo, Cerebro de rata.

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