MELATONIN, NITRIC OXIDE SYNTHASE AND OXIDATIVE STRESS: IMPLICATIONS IN AGING

D.K. Lahiri, Y-.W. Ge, J.I. Nurnberger, Jr., E.Y. Yang, S.C. Bondy[1]

1. INTRODUCTION

Age-related changes in cerebral functions are believed to be partially due to an imbalance between pro-oxidant and antioxidant factors.[1] To compensate for this imbalance, dietary supplementation with various antioxidant agents is being tested.[2] We have been studying the neuroprotective and biochemical role of melatonin.[3] Melatonin, an indoleamine derivative of serotonin, is synthesized within the pineal body, and melatonin levels in the plasma have been noted to decrease with age.[4] The effects of addition of melatonin to the diet over an extended time have been studied with emphasis on age-related changes in two indices of oxidant activity: the rate of formation of reactive oxygen species (ROS) and the activity of nitric oxide synthase (NOS). Ages of mice that reflected middle adulthood and pre-senescent periods rather than stages of early development or extreme age were selected.

2. METHODS

2.1. Animals and Diets

Male B/6C3F1 mice, a hybrid between C57BL/6 and C3H from Harlan Labs, Indianapolis, IN, were maintained on a 12 hour light/ dark cycle in a temperature controlled (20±1°C) room.[2] Diets and water were provided ad lib. The minimal basal diet (#101101, Dyets Inc., PA) consisted of 50% sucrose and 26% casein (w/w) with a minimal salt and vitamin mix that include 110 ppm α-tocopherol acetate. This diet was supplemented with 0.004% melatonin and diets were fed to mice from age 3 to 9 months.

[1] D.K. Lahiri, Y-.W. Ge, J.I. Nurnberger, Jr., nstitute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive Indianapolis, IN 42602; E.Y. Yang, S.C. Bondy, Department of Community and Environmental Medicine, University of California, Irvine, CA-92697. [1] D.K. Lahiri- Corresponding author

Catecholamine Research, Edited by Nagatsu *et al.*
Kluwer Academic/Plenum Publishers, 2002

2.2. Assay of NOS activity in mice brain extracts

NOS activity from the mice cerebellum was assayed using L-[^{3}H]-arginine (Amersham, NJ) and a kit (Stratagene, CA) as described previously.[5] A ratio of radioactive citrulline to unreacted arginine gives the percent conversion of arginine to citrulline and reflects a measure of net NOS activity under the condition used here.

2.3. Measurement of the rate of formation of reactive oxygen species

The cerebrocortical synaptosomal/ mitochondrial fraction was prepared to measure the rate of formation of ROS using 2',7'-dichlorofluorescin diacetate (DCFH-DA).[6] Briefly, DCFH-DA is de-esterified within cells to the ionized free acid, which was oxidized to the fluorescent 2',7'-dichlorofluorescein (DCF) by ROS[6].

3. RESULTS

3.1 Optimization of NOS activity in mouse brain extracts

The assay used was based on monitoring the biochemical conversion of L-arginine to L-citrulline by NOS. This reaction involves a five-electron oxidation of the guanidino-nitrogen of L-arginine to nitric oxide, together with the stoichiometric production of L-citrulline. The reaction requires NADPH, molecular oxygen, calcium, calmodulin and tetrahydrobiopterin. Since this assay is based on the use of radioactive arginine, the sensitivity of the assay is in the picomole level. The standard curve for the NOS assay indicates that NOS activity was linear with 5 to 30 μg of cerebellar extracts in our laboratory. Protein samples within the linear range of NOS activity were used.

3.2. Effect of age and dietary supplementation with melatonin on NOS activity

The activity of NOS in different treatment groups was compared (Table 1). The activity was greatly reduced in older 9-month mice (46.3%) relative to younger 3-month mice. When younger mice were fed a melatonin supplemented diet (40 ppm) for 6 months the original activity was almost completely maintained (87.5%) (Table 1). Results were very similar when NOS activity was adjusted to per mg wet weight of the original tissue. In this case, NOS activity was reduced to 51.4 % in older mice relative to corresponding values for younger mice, and the original activity was maintained to 98.3% when younger mice were fed diets supplemented with melatonin for 6 months.

Table 1. Assay of the activity of NOS in mice cerebellar extracts[a]

Age of mice	Treatment	NOS (% conversion)[b]	S.E.M	% original activity
3 months	None	7.98	0.87	100
9 months	None	3.69*	0.51	46.3
9 months	Melatonin	7.01	1.06	87.5

[a] NOS activity is expressed as the percent conversion of arginine to citrulline per 10 μg of protein samples. Seven animals were used/group.

[b] * Value differs significantly from corresponding value for 3 month-old mice receiving basal diet.

Table 2. Measurement of the formation of ROS[a]

Age of mice	Treatment	ROS [b]	S.E.M	% activity
3 months	None	422.8	2.18	100
9 months	None	294.3*	1.31	69.6
9 months	Melatonin	412.7	3.17	97.6

[a] The rate of formation of ROS was quantitated using a DCF standard curve and results were expressed as nmol DCF formed/ mg protein/ hour. Seven animals were used/group.

[b] * Value differs significantly from corresponding value for 3 month-old mice receiving basal diet.

3.3. Effect of age and dietary supplementation with melatonin on ROS generation

A similar trend to that of NOS activity was observed when the rate of generation of reactive oxygen species within cerebral cortex was measured. However, the age-related decline of ROS was somewhat less than was the case with NOS. Melatonin supplementation in the diets for 6 months was able to maintain the rate of ROS formation to 97.6% of the corresponding 3 month-old value (Table 2).

4. DISCUSSION

The goal of this work was to study the alteration of cerebral redox status during aging, and to use a specific dietary supplement in an attempt to maintain oxidant parameters at levels paralleling those in younger animals. A major age-related decrease in the rate of ROS generation and NOS activity was found but addition of melatonin to the basal diet restored both NOS and ROS generation rates to levels not significantly different from those found in younger animals. These data suggest that melatonin supplementation may result in maintaining a younger metabolic rate profile in chronologically older mice.

Because of its ability to retard age-related changes, melatonin supplementation may have potential value in the treatment of neurological disease closely associated with senescence such as Alzheimer's disease (AD). This is supported by reports that melatonin may play a role in several neuropsychiatric conditions.[7, 8] Abnormalities in melatonin secretion are found in AD[7] and bipolar I affective disorder subjects.[8] It is possible that dietary supplementation of melatonin would retard the progressive imbalance of homeostatic cellular mechanisms characterizing these disorders. The reason to select 3- and 9-month old mice was to avoid age-related extremes but also to determine the changes associated with transition from full maturity to early aging. Major reductions in both NOS levels and ROS production rate occurred with aging despite the relatively short age span covered. The current findings are in contrast to other reports of increased levels of oxidative damage to macromolecules in aging brain.[9] This may be due to the fact that these latter results represented a summation of events over an extended duration. In contrast, our assays reflect a snapshot of actual, ongoing dynamic pro-oxidant status in the aging animal at death, a single instant in time. The present findings are consistent with an earlier report of an age-related depression of short-lived oxidative species.[1]

The reason to select measurement of redox processes, such as ROS generation rate and NOS activity, as biochemical endpoints is because of their involvement in maintaining cellular homeostasis in the body. The major source of the intracellular

formation of ROS is believed to be mitochondria. Therefore, reductions of both ROS formation rate and NOS activity in aged mice may reflect a slowing of the functioning of the electron transport chain with age as also shown in another study.[10] Melatonin supplementation was chosen because this agent can readily traverse the blood-brain barrier and some data suggest that it is neuroprotective[4]. Our present results argue strongly for a neuroendocrine role of melatonin during aging. Thus the increase in ROS and NOS found in melatonin-treated older animals may reflect maintenance of levels of mitochondrial activity characterizing that of younger animals, rather than implying deleterious events.

5. CONCLUSION

Levels of cerebellar NOS and rates of generation of cortical ROS were significantly reduced in 9 month-old mice relative to 3 month-old mice. Since most intracellular ROS have a mitochondrial origin, this may reflect a slowing down of the electron transport chain with age. Following six months treatment with dietary melatonin, the NOS activity and ROS formation rate were restored to the corresponding values found in the younger group of mice. Melatonin's ability to maintain the original levels of ROS and NOS may be due to increased endocrine function, rather than any direct antioxidant property. The dietary presence of melatonin may help maintain oxidant parameters at levels characteristic of younger animals by compensating for some of the loss of mitochondrial activity during aging.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (ES 7992 and AG 16794) and the Indiana Division of Mental Health.

REFERENCES

1. LeBel CP and Bondy SC. Oxidative damage and cerebral aging. *Prog. Neurobiol.* **38**, 601-602 (1992).
2. Bondy SC, Yang YE, Walsh TJ, Ge Y and Lahiri DK. Dietary modulation of age-related changes in cerebral pro-oxidant status. *Neurochem. Int.*- In Press
3. Lahiri DK and Ghosh C. Interactions between melatonin, reactive oxygen species(ROS) and nitric oxide. *Ann. N.Y. Acad. Sci.* **893**, 325-330 (1999).
4. Reiter RJ. The role of the neurohormone melatonin against macromolecular oxidative damage. *Neurochem. Int.* **6**, 453-460 (1995).
5. Ghosh C and Lahiri DK. Increased vulnerability of neuronal cell lines to sodium nitroprusside-mediated toxicity is caused by the decreased level of nitric oxide metabolites. *J. Mol. Neurosci.* **13**, 77-92 (1999).
6. Bass DA, Parce JW, Dechatelet LR, et al. Flow cytometric studies of oxidative product formation by neutrophils: A graded dose response to membrane stimulation. *J. Immunol.* **130**, 1910 (1983).
7. Witting W, Kwa IH, Eikelenboom P, Mirmiran M and Swaab DF. Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. *Biol. Psychiat.* **27**, 563-572 (1990).
8. Nurnberger Jr, JI, Adkins S, Lahiri DK, Mayeda A, et al.. Melatonin suppression by light in euthymic bipolar and unipolar patients. *Arch. Gen. Psychiat.* **57**, 572-579 (2000).
9. Smith CD, Carney JM, Starke-Reed PE, et al.. Excess protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. *Proc. Natl. Acad. Sci.* **88**, 10540-10543 (1991).
10 Sharman EH and Bondy SC. Effects of age and dietary antioxidants on cortical electron transport chain activity. *Neurobiol. Aging* **22**, 629-634 (2001).

CATECHOLAMINE RESEARCH

From Molecular Insights to Clinical Medicine

Edited by

Toshiharu Nagatsu
Fujita Health University
Toyoake, Aichi, Japan

Toshitaka Nabeshima
Nagoya University
Nagoya, Japan

Richard McCarty
Vanderbilt University
Nashville, Tennessee

and

David S. Goldstein
National Institute of Neurological Disorders and Stroke
Bethesda, Maryland

Springer Science+Business Media, LLC

Catecholamine research: from molecular insights to clinical medicine/edited by Toshiharu Nagatsu ... [et al.].
p. cm. — (Advances in behavioral biology; 53)
Includes bibliographical references and index.

1. Catecholamines—Congresses. I. Nagatsu, Toshiharu. II. Series.

QP801.C33 C383 2002
612.8'042—dc21

2002028277

Proceedings of the 9th International Catecholamine Symposium, held from March 31st to April 5th, 2001, in Kyoto, Japan

ISBN 978-1-4419-3388-1 ISBN 978-1-4757-3538-3 (eBook)
DOI 10.1007/978-1-4757-3538-3

Originally published by Kluwer Academic / Plenum Publishers, New York in 2002.

Softcover reprint of the hardcover 1st edition 2002

http://www.wkap.nl/

10 9 8 7 6 5 4 3 2 1

A C.I.P. record for this book is available from the Library of Congress

Catecholamine Research: From Molecular Insights to Clinical Medicine (Advances in Behavioral Biology) 2002nd Edition

by Toshiharu Nagatsu (Editor), Toshitaka Nabeshima (Editor), Richard McCarty (Editor),

Look inside

ISBN-13: 978-0306474033
ISBN-10: 0306474034
Why is ISBN important?

Have one to sell? Sell on Amazon

Submit

Share

Kindle	Hardcover	Paperback	Other Sellers
$63.80 - $328.60	$42.00 - $345.99	$345.90	from $42.00

Buy used $42.00

Buy new **$345.99**

In stock.
Ships from and sold by allnewbooks.

4 New from $345.99

Select delivery location

1

Get it as soon as Jan. 8 - 15 when you choose **Standard Shipping** at checkout.

$345.99 + $3.99 shipping

Add to Cart

Submit

More Buying Choices

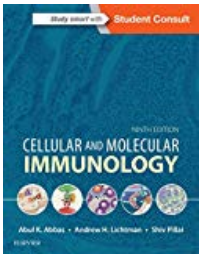
11 used & new from $42.00

4 New from $345.99 | 7 Used from $42.00

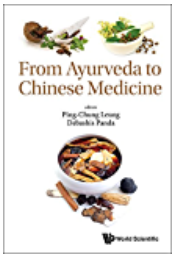
See All Buying Options

See the Best Books of 2019
Browse the Amazon editors' picks for the Best Books of 2019, featuring our favorite reads in more than a dozen categories.

Sponsored products related to this item



Cellular and Molecular Immunology
Abul K Abbas Mbbs



FROM AYURVEDA TO CHINESE MEDICINE
Ping-Chung Leung

Critical Decisions in Emergency Medicine: November, 2017

Critical Decisions in Emergency Medicine: September, 2017

Critical Decisions in Emergency Medicine: June, 2017

Product details

Series: Advances in Behavioral Biology (Book 53)
Hardcover: 558 pages
Publisher: Springer; 2002 edition (October 31, 2002)
Language: English
ISBN-10: 0306474034
ISBN-13: 978-0306474033
Product Dimensions: 7 x 1.2 x 10 inches
Shipping Weight: 2.8 pounds (View shipping rates and policies)
Average Customer Review: Be the first to review this item
Amazon Best Sellers Rank: #10,424,250 in Books (See Top 100 in Books)
#5769 in Neuroscience (Books)
#4188 in Pharmacy (Books)
#4679 in Cardiology (Books)

Would you like to **tell us about a lower price**?
If you are a seller for this product, would you like to **suggest updates through seller support**?

Related video shorts (0) Upload your video

Be the first video
Your name here

No customer reviews

5 star	0%
4 star	0%
3 star	0%
2 star	0%
1 star	0%