Experimental Gerontology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short report

Light–dark condition regulates sirtuin mRNA levels in the retina

Norimitsu Ban [a,b], Yoko Ozawa [a,b,*], Takaaki Inaba [b], Seiji Miyake [a,b], Mitsuhiro Watanabe [c,d], Ken Shinmura [c], Kazuo Tsubota [b]

[a] Laboratory of Retinal Cell Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
[b] Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
[c] Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
[d] Graduate School of Media and Governance, Faculty of Environment and Information Studies, Keio University, 5322 Endo, Fujisawa, Kanagawa 252-0882, Japan

ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form 3 April 2013
Accepted 24 April 2013
Available online xxxx

Section Editor: Christian Humpel

Keywords:
Sirtuins
Retina
Light
Nampt
Mitochondria
Metabolism

ABSTRACT

Sirtuins (Sirt1–7) are nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases/ADP-ribosyltransferases that modulate many metabolic responses affecting aging. Sirtuins expressed in tissues and organs involved in systemic metabolism have been extensively studied. However, the characteristics of sirtuins in the retina, where local energy expenditure changes dynamically in response to light stimuli, are largely unknown. Here we analyzed sirtuin mRNA levels by real-time PCR, and found that all seven sirtuins are highly expressed in the retina compared with other tissues, such as liver. We then analyzed the sirtuin mRNA profiles in the retina over time, under a 12-h light/12-h dark cycle (LD condition) and in constant darkness (DD condition). All seven sirtuins showed significant daily variation under the LD condition, with all except Sirt6 being increased in the dark phase. The expression patterns were different under the DD condition, suggesting that sirtuin mRNA levels except Sirt6 are affected by light–dark condition. These findings were not obtained in the brain and liver. In addition, the mRNA expression patterns of Nicotinamide phosphoribosyltransferase (Nampt), peroxisome proliferator-activated receptor gamma coactivator (PGC1α), and transcription factor A, mitochondrial (Tfam) in the retina, were similar to those of the sirtuins except Sirt6. Our observations provide new insights into the metabolic mechanisms of the retina and the sirtuins' regulatory systems.

1. Introduction

Sirtuins are evolutionarily conserved nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases/ADP-ribosyltransferases that contribute to metabolic and stress responses and affect aging (Satoh et al., 2011). All seven mammalian sirtuin homologues (Sirt1–7) are involved in energy metabolism. Sirt1, Sirt6, and Sirt7, the nuclear sirtuins, regulate the activity of key transcription factors and cofactors of numerous metabolic pathways, linking nutrient signals with cellular responses to energy demands (Chalkiadaki and Guarente, 2012). The mitochondrial sirtuins, Sirt3, Sirt4, and Sirt5, regulate the activity of mitochondrial enzymes and drive metabolic cycles in response to fasting and calorie restriction (Chalkiadaki and Guarente, 2012). Sirt2, which is primarily cytoplasmic, has roles in fatty acid oxidation, energy expenditure regulation (Krishnan et al., 2012), and cell-cycle control (Dryden et al., 2003). Under conditions of high energy demand, the level of the sirtuins' co-factor, NAD, increases, and the sirtuins are activated; consequently, their target molecules are enzymatically modified to provide energy. The sirtuins are well documented in tissues and organs involved in systemic metabolism (e.g., the liver and heart). However, the sirtuins in the retina, where local energy expenditure dynamically changes in response to light stimuli (Ames et al., 1992), remain largely uncharacterized (Ozawa et al., 2010).

In the retina, visual pigment receives and converts light energy to an electric impulse that is transmitted to and processed in the neuronal tissue of the retina, leading to visual perception in the visual cortex of the brain. Thus, the energy consumption changes greatly according to the neuronal activity in the retina, which depends on light stimuli (Ames et al., 1992; Niven and Laughlin, 2008). Although metabolic control is essential for vision, it is not known whether all seven sirtuins are expressed in the retina.

In this study, we analyzed the transcript levels of Sirt1–7 in the retina and the effects of light–dark conditions on their expression levels and on the mRNA for Nicotinamide phosphoribosyltransferase (Nampt), which controls NAD production. Moreover, we also analyzed the levels of the mRNAs for peroxisome proliferator-activated receptor gamma coactivator (PGC1α) and transcription factor A, mitochondrial (Tfam), which are indicators of mitochondrial biogenesis;

Abbreviations: NAD, nicotinamide adenine dinucleotide; Nampt, nicotinamide phosphoribosyltransferase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator; Tfam, transcription factor A, mitochondrial; LD condition, light/dark condition; DD condition, constant dark condition; ZT, zeitgeber time; CT, circadian time.

* Corresponding author at: Laboratory of Retinal Cell Biology, Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan. Tel.: +81 3 3353 1211; fax: +81 3 3359 8302.
E-mail address: ozawa@a5.keio.jp (Y. Ozawa).

0531-5565/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.exger.2013.04.010

mitochondrial biogenesis is enzymatically regulated by Sirt1 (Rodgers et al., 2005). We compared the profiles of sirtuin mRNA levels over time in mice kept under a 12-h light/12-h dark (LD) cycle with their levels in mice kept in constant darkness (DD). Our findings provide basic information about the sirtuins in the retina, which will be essential for future research on retinal metabolism and sirtuin regulation.

2. Materials and methods

2.1. Animals and tissue sampling

Six-week-old male C57BL6/J mice (CLEA Japan, Tokyo, Japan) were used. The mice were kept in an air-conditioned room (22 ± 1 °C) under a 12-h light/12-h dark (LD) cycle, with the light on from 08:00 to 20:00. The light intensity at the surface of the cages was 200 lx. The mice were given food and water ad libitum. After being subjected to the LD cycle for 2 weeks, the mice were randomly divided into an LD group and a constant darkness group (DD group). The LD group was kept under the LD cycle and sacrificed at zeitgeber time (ZT) 0, 4, 8, 12, 16, and 20 h. ZT0 was defined as the time of light onset and ZT12 as the time of dark onset. The DD group was transferred to constant darkness after the dark phase of the last LD cycle, and kept in the dark for two 24-hour DD cycles. During the last and third 24-hour DD cycles, the mice were sacrificed at circadian time (CT) 0, 4, 8, 12, 16, and 20 h. CT0 was defined as the onset time of the last 24-hour DD cycle. Five or 6 mice were sacrificed at each time point, and samples of the retina, brain parietal cortex, heart, liver, and kidney were obtained. For tissue sampling during the dark phase, a dim red light was used, and the samples were placed individually in light-tight containers on ice and dissected under a microscope within 2 min. All the animal experiments were conducted in accordance with the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. RNA isolation and real-time PCR

The retina or other tissue sample was placed in TRIzol reagent (Life Technologies, Carlsbad, CA, USA) to extract the total RNA. To generate complementary DNA (cDNA), 1 μg of the total RNA was added to the Super Script VILO cDNA Synthesis Kit (Life Technologies) and reverse-transcribed according to the manufacturer's instructions. PCR was performed using the StepOnePlus Real Time PCR system (Life Technologies), and the mRNA was quantified using the delta delta CT method. To compare the mRNA levels in the retina with those in other tissues, the expression levels were normalized to that for β-actin. To obtain profiles of the mRNA levels over time, the expression levels were normalized to that for 36B4, which showed constant expression under LD and DD conditions (data not shown). The primers used in this study are shown in Table 1.

2.3. Statistics

Results are presented as the mean ± SD, and $p < 0.05$ was considered statistically significant. To compare the sirtuin expression levels among tissues, we used Tukey's HSD test or the Games–Howell test, depending on Levine's test for equality of variance. The mRNA expressions over time were compared by one-way ANOVA within the LD or DD group, followed by Tukey's HSD test or the Games–Howell test. The expression levels between LD and DD groups at each time point for 24 h were compared by Student's *t*-test. All statistical tests were performed using IBM SPSS statistics Ver.19 (IBM, Armonk, NY).

Table 1
Primer sequences.

Gene	Primer sequence 5′ to 3′ Forward and reverse
Sirt1	ACTCCTCACTAATGGCTTTCATTC
	GGTGGAGGAATTGTTTCTGGTAAT
Sirt2	CCTCTGACCCTCTGGAGACC
	AAGACGCTCCTTTTGGGAAC
Sirt3	TACAGGCCCAATGTCACTCA
	CTTCGACAGACCGTGCATGTA
Sirt4	GTCGTTTTCTTTGGGGACAC
	AGAATGGCTATTGGGAGCTTTT
Sirt5	AGCAAGATCTGCCTCACCAT
	GCCTGCCATTTTCTCCAGTA
Sirt6	AGGCCGTCTGGTCATTGTC
	GCACATCACCTCATCCACGTA
Sirt7	AGCCTACCCTCACCCACATG
	GGTGGAGCCCATCACAGTTC
Nampt	TCAAGGAGATGGCGTGGATA
	CACCAGAACCGAAGGAGACA
PGC1α	GATGAATACCGCAAAGAGCA
	AGATTTACGGTGCATTCCTCA
Tfam	AGTCAGCTGATGGGTATGGAGAA
	TGCTGAACGAGGTCTTTTTGG
β-actin	AGGTCATCACTATTGGCAACGA
	GTTTCATGGATGCCACAGGA
36B4	CGACCTGGAAGTCCAACTAC
	ATCTGCTGCATCTGCTTG

3. Results

3.1. High sirtuin mRNA levels in the retina

We first analyzed the mRNA levels of sirtuins in the retina and other tissues (brain parietal cortex, heart, liver, and kidney) at ZT20 by real-time PCR (Fig. 1). The mRNA levels of Sirt1, 2, 4, 6, and 7 were significantly higher in the retina than in all the other tissues examined. The Sirt3 mRNA level in the retina was the same as in the liver, and significantly higher than in the rest of the tissues. The Sirt5 mRNA level in the retina was significantly higher than in all the other tissues examined except the heart (relative mRNA levels were; retina: brain: heart: liver: kidney, Sirt1 1.0: 0.15: 0.22: 0.09: 0.18, Sirt2 1.0: 0.58: 0.52: 0.46: 0.50, Sirt3 1.0: 0.58: 0.66: 1.0: 0.64, Sirt4 1.0: 0.08: 0.16: 0.08: 0.09, Sirt5 1.0: 0.26: 0.88: 0.37: 0.30, Sirt6 1.0: 0.15: 0.24: 0.04: 0.13, Sirt7 1.0: 0.34: 0.26: 0.47: 0.34).

3.2. The mRNA profiles of sirtuins in the retina under LD and DD conditions over time

We next analyzed the mRNA profiles of sirtuins in the retina, brain, and liver over the course of a day under LD and DD conditions, by real-time PCR (Fig. 2). In the retina, all the sirtuins (Sirt1–7) showed significant daily variation under the LD condition (ANOVA: Sirt1–7 $p < 0.01$). The mRNA levels of all the sirtuins except Sirt6 were significantly increased in the dark phase compared with ZT0, peaking at ZT16–ZT20 (Sirt1 1.6 fold at ZT20; Sirt2 1.7 fold at ZT 20; Sirt3 1.8 fold at ZT 16; Sirt4 1.9 fold at ZT 20; Sirt5 1.7 fold at ZT20; Sirt7, 1.9 fold at ZT20).

In contrast, under the DD condition, none of the sirtuins was elevated or peaked during CT16–CT20, the comparative peak time under the LD condition. Moreover, the mRNA levels of all the sirtuins except Sirt6 were significantly higher at each time point from CT0 to CT12 under the DD condition than under the LD condition from ZT0 to ZT12 (relative mRNA levels of CT0 compared to ZT0; Sirt1 2.3 fold; Sirt2 2.5 fold; Sirt3 2.0 fold; Sirt4 2.2 fold; Sirt5 2.0 fold; Sirt7 2.2 fold). Regarding the daily variations under the DD condition, those of Sirt2–5 were significant (ANOVA: Sirt2, 4, and 5 $p < 0.01$; Sirt3 $p = 0.028$), but those of Sirt1, 6, and 7 were not.

Please cite this article as: Ban, N., et al., Light–dark condition regulates sirtuin mRNA levels in the retina, Exp. Gerontol. (2013), http://dx.doi.org/10.1016/j.exger.2013.04.010