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Research paper

Specific visible radiation facilitates lipolysis in mature 3T3-L1 adipocytes *via* rhodopsin-dependent β3-adrenergic signaling

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ABSTRACT

The regulation of fat metabolism is important for maintaining functional and structural tissue homeostasis in biological systems. Reducing excessive lipids has been an important concern due to the concomitant health risks caused by metabolic disorders such as obesity, adiposity and dyslipidemia. A recent study revealed that unlike conventional care regimens (*e.g.*, diet or medicine), low-energy visible radiation (VR) regulates lipid levels *via* autophagy-dependent hormone-sensitive lipase (HSL) phosphorylation in differentiated human adipose-derived stem cells. To clarify the underlying cellular and molecular mechanisms, we first verified the photoreceptor and photoreceptor-dependent signal cascade in non-visual 3T3-L1 adipocytes. For a better understanding of the concomitant phenomena that result from VR exposure, mature 3T3-L1 adipocytes were exposed to four different wavelengths of VR (410, 505, 590 and 660 nm) in this study. The results confirmed that specific VR wavelengths, especially 505 nm than 590 nm, increase intracellular cyclic adenosine monophosphate (cAMP) levels and decrease lipid droplets. Interestingly, the mRNA and protein levels of the Opn2 (rhodopsin) photoreceptor increased after VR exposure in mature 3T3-L1 adipocytes. Subsequent treatment of mature 3T3-L1 adipocytes at a specific VR wavelength induced rhodopsin- and β3-adrenergic receptor (AR)-dependent lipolytic responses that consequently led to increases in intracellular cAMP and phosphorylated HSL protein levels. Our study indicates that photoreceptors are expressed and exert individual functions in nonvisual cells, such as adipocytes. We suggest that the VR-induced photoreceptor system could be a potential therapeutic target for the regulation of lipid homeostasis in a non-invasive manner.

1. Introduction

The regulation of fat metabolism, including both the biosynthesis and degradation of fat, is important for maintaining lipid homeostasis in biological systems. Excess lipid accumulation due to the dysregulation of fat metabolism increases the risks of metabolic disorders such as obesity, cardiovascular disease and type 2 diabetes (Peirce et al., 2014). Although most cells are involved in this metabolism, adipocytes play important roles in fat storage, mobilization and degradation. Specifically, white adipocytes are particularly critical in subcutaneous and visceral regions compared with brown adipocytes and beige adipocytes (Cannon and Nedergaard, 2004; Hansen and Kristiansen, 2006; Wu et al., 2012).

Lipolysis in adipocytes is a major metabolic process in which fat is broken down into free glycerides and fatty acids, and this process is facilitated by cyclic adenosine monophosphate (cAMP) through β-adrenergic receptors (ARs) (Kraemer and Shen, 2002; Hallows et al., 2009; Krintel et al., 2009). Several non-adrenergic pathways are also known to be involved in lipolysis, which raises the possibility that other regulatory factors, such as non-adrenal stimuli, bioactive compounds, and physical stimulators, may be involved in lipolysis (Villarroya and Vidal-Puig, 2013).

Photo-regulation using ultraviolet (UVR, 280–400 nm), visible (VR, 400–760 nm) and infrared radiation (IR, 760 nm–1 mm) has emerged in dermatological fields in association with pigmentation, aging, carcinogenesis and epidermal hyperplasia (Lee et al., 2002; Schieke et al., 2003; Demidova-Rice et al., 2007; Mahmoud et al., 2010; Ramasubramaniam et al., 2011; Sklar et al., 2013). Compared with other forms of radiation, VR has generally been shown to be beneficial in the treatment of human skin diseases and the allevia-

Abbreviations: VR, visible radiation; Opn1, cone opsin; Opn2, type II opsin; β2-AR, beta-2 adrenergic receptor; β3-AR, beta-3 adrenergic receptor type 3; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; HSL, hormone-sensitive lipase; PDE, phosphodiesterase; LED, light-emitting diode.

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tion of concomitant adverse effects such as pain and inflammation (Bernstein, 2005; Triesscheijn et al., 2006; Avci et al., 2013; Gupta et al., 2014). However, the blue spectrum (450–490 nm) has been reported to exert various genotoxic effects and to generate reactive oxygen species (ROS), and the violet spectrum (380–420 nm) has been reported to modulate the differentiation of keratinocytes (Sideris et al., 1981; Pflaum et al., 1998; Omata et al., 2006; Botta et al., 2008; Kim et al., 2013). These studies suggest that photoregulation using a specific/narrow range of wavelengths (and not the full range) is needed to minimize the adverse effects associated with visible light. More importantly, the recipient cells in local regions, especially human epidermal melanocytes and keratinocytes, should respond to the VR by expressing opsins (Opns), mainly rhodopsin (Opn2) and encephalopsin (Opn3) (Garriga and Manyosa, 2002; Wicks et al., 2011; Haltaufderhyde et al., 2015). These findings suggest that OPNs could act as photosensors even in normal nonvisual cells beneath the skin. Despite the various effects of VR, a precise photo-regulatory mechanism mediated by specific VRs in adipocytes remains unknown.

The aim of our study is to investigate the status of photoreceptors and to additionally verify their lipolytic effect in adipocytes following exposure to specific low-energy visible radiation (VR). Specifically, we focus on the existence of rhodopsin and rhodopsin-mediated lipolysis in adipocytes that result from the intracellular accumulation of cyclic AMP (cAMP) *via* the β3-adrenergic receptor (AR) and phosphorylated hormone-sensitive lipase (HSL) following exposure to specific VR. Based on the correlation of photoreceptors with β3-AR activation, we suggest that the VR-induced photoreceptor system could be a potential therapeutic target for the regulation of lipid homeostasis in a non-invasive manner.

2. Materials and methods

2.1. Cell culture and differentiation

Mouse 3T3-L1 adipocytes (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Lonza, Walkersville, MD, USA) supplemented with 10% calf serum (Gibco BRL, NY, USA) in a humid 10% CO_2 incubator. To induce differentiation, 3T3-L1 adipocytes were sequentially replenished with the following media: 1) DMEM containing 10% fetal bovine serum (FBS; PAA, Pasching, Austria), 10 μg/ml insulin (Sigma-Aldrich, St. Louis, MO, USA), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich), and 1 μM dexamethasone (DEX; Sigma-Aldrich) for 2 days; 2) DMEM supplemented with 10% FBS and 10 μg/ml insulin for 3 days; and 3) DMEM containing 10% FBS without insulin for 2 days.

2.2. Visible radiation

An LED irradiation system was used according to the manufacturer's instructions (HanaroTR, Suwon, Korea), and each LED array emitted a particular wavelength as previously described and as follows (Kim et al., 2013): violet LED (380–420 nm, λ_{max}: 410 nm), green LED (450–560 nm, λ_{max}: 505 nm), orange LED (560–620 nm, λ_{max}: 590 nm), and red LED (620–690 nm, λ_{max}: 660 nm). For the irradiation, the mouse 3T3-L1 adipocytes were sequentially differentiated for 7 days in a 10% CO_2 incubator using the different medium conditions described above and then briefly rinsed twice with phosphate-buffered saline (PBS, Welgene, Daegu, Korea). The cells were replenished with fresh PBS, moved inside a dark chamber with 10% CO_2 at 37 °C and subsequently then irradiated with each LED light at 36 J/cm^2 (10 mW/cm^2 for 60 min) in a darkroom. After irradiation for 1 h, the cells were replenished with serum-free medium and incubated in a darkroom for the indicated time periods.

2.3. Determination of the glycerol content

The released glycerol was measured with a free glycerol determination kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. The culture medium was harvested 24 h after irradiation at the selected wavelengths. The data are presented as the percentages relative to either the untreated control cells or the glycerol concentrations.

2.4. Oil Red O staining

The cells were washed twice with cold PBS and fixed with 3.7% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 1 h. The fixed cells were washed with 60% propylene glycol (Sigma-Aldrich, St. Louis, MO, USA) in PBS and were stained with a working solution of Oil Red O (0.3% Oil Red O in 60% propylene glycol; Sigma-Aldrich, St. Louis, MO, USA) for 30 min. The cells were washed with 85% propylene glycol thrice and rinsed with tap water. Lipid droplets stained with Oil Red O dye were visualized with an IX71 microscope (Olympus, Tokyo, Japan).

2.5. Analysis of the total glycerol content

The total glycerol (TG) content in the cells after VR irradiation was measured *via* the Sudan-II staining method (Sigma-Aldrich, St. Louis, USA). The cells were washed twice with ice-cold PBS, fixed with 3.7% formaldehyde for 20 min, and then stained with 0.5% Sudan II in 60% isopropyl alcohol (Sigma-Aldrich, St. Louis, USA) for 1 h. After gentle washing with 70% ethyl alcohol (Sigma-Aldrich, St. Louis, USA), the TG-attached Sudan II was extracted with 4% NP-40 (Sigma-Aldrich, St. Louis, USA) in isopropyl alcohol for 20 min, and the absorbances were measured at 490 nm using a Synergy H2 reader (BioTek., VT, USA).

2.6. cAMP assay

The cells were harvested at various time points after VR irradiation using 0.1 mM hydrochloric acid (Sigma-Aldrich, St. Louis, MO, USA) after being washed twice with cold PBS. After centrifugation at 600 × *g* for 10 min at 4 °C, the cAMP levels in the supernatants were measured using a cAMP assay kit according to the manufacturer's instructions (BioVision, Milpitas, CA, USA).

2.7. Western blot analysis

The cells were harvested with RIPA buffer (Thermo Scientific Co., Pittsburgh, PA, USA) containing protease inhibitors (Sigma-Aldrich, St. Louis, MO, USA). The proteins (20 μg) were loaded, transferred, and probed with the following antibodies: (a) anti-ADRB2 (β2-AR: bs-0947R) and anti-ADRB3 (β3-AR: bs-1063R), which were purchased from Bioss antibodies (Woburn, MA, USA); (b) anti-protein kinase A (PKA: sc-903), anti-phospho-PKA (sc-32968), and anti-gamma tubulin (γTUB: sc-7396), which were purchased from Santa Cruz Biotechnology; and (c) anti-rhodopsin (Rho: #8710), anti-protein kinase G (PKG: #3248), anti-phospho-PKG (#3114), anti-hormone-sensitive lipase (HSL: #4107), and anti-phospho-HSL (#4139), which were purchased from Cell Signaling Technology (Danvers, MA, USA). The data presented for the western blot analyses represent repetitions of the experiments using biologically different samples (n = 3). The relative intensity of each band on western blot analyses was measured using Image J software.