Journal of Thermal Biology 74 (2018) 256–263

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Short-term heat stress induces mitochondrial degradation and biogenesis and enhances mitochondrial quality in porcine oocytes

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ARTICLE INFO

Keywords:
Oocyte
Short-term heat stress
Mitochondria
HSP72
SIRT1

ABSTRACT

Mitochondria in oocytes play important roles in many processes, including early embryo development. Promotion of mitochondrial degradation and biogenesis through Sirtuin 1 (SIRT1) activation enhances mitochondrial function and oocyte quality. Previous studies that used somatic cells have shown that short-term heat stress (SHS) induces SIRT1-regulated mitochondrial biogenesis. In this study, we examined whether SHS can induce mitochondrial degradation and biogenesis in porcine oocytes. We collected cumulus cell-oocyte complexes (COCs) from prepubertal gilt ovaries acquired from a slaughterhouse. COCs were treated at 41.5 °C (vehicle: 38.5 °C) for the first one hour of in vitro maturation, and the mitochondrial kinetics, oocyte function, and developmental competence of oocytes were examined. SHS increased the expression level of heat shock protein 72, which induced the high expression of SIRT1 and the phosphorylation of AMP-activated protein kinase. SHS did not alter the mitochondrial DNA copy number in oocytes, but induced mitochondrial degradation and biogenesis, which enhanced the mitochondrial membrane potential and ATP content in oocytes, and improved the ability of the oocytes to develop into blastocysts.

1. Introduction

In somatic cells, mitochondria play crucial roles in calcium accumulation, apoptosis, and energy production. In domestic animals and humans, an oocyte contains about 100,000 mitochondria. The mitochondria supply ATP to the oocytes through fatty acid oxidation and oxidative phosphorylation for oocyte growth, maturation, fertilization, and early embryo development (Reynier et al., 2001; Dumollard et al., 2004; Santos et al., 2006; Dunning et al., 2010; Iwata et al., 2011). The mitochondrial number is believed to be crucial to the extent that oocytes containing a low number and low quality of mitochondria have a low oocyte quality (May-Panloup et al., 2005; Zhang et al., 2006). Therefore, it is important to control the mitochondrial quality and quantity in oocytes for use in assisted reproduction technology in humans and animals.

We previously reported that mitochondrial uncoupler (CCCP)-induced mitochondrial depolarization in porcine oocytes leads to Sirtuin 1 (SIRT1: histone deacetylase)- and AMP-activated protein kinase (AMPK)-mediated mitochondrial degradation and biogenesis, and maintains both the mitochondrial quality and the ability of oocytes to develop into blastocysts (Itami et al., 2015). Furthermore, we also showed that resveratrol, a potent and specific activator of SIRT1, promotes mitochondrial degradation and biogenesis, and enhances the developmental ability of oocytes (Sato et al., 2014). These findings indicate that non-invasive activation of SIRT1 is effective in improving the quality of mitochondria, as well as the developmental ability of oocytes.

Karpe and Tikoo (2014) reported that short-term heat stress (SHS) in rats reverses the high fat diet-induced low expression of SIRT1 in aortic tissues. They further suggested that high SIRT1 expression in tissues is derived from heat stress-induced activation of heat shock protein 72 (HSP72). Henstridge et al. (2014) showed that mice with HSP72 overexpression display activation of SIRT1 and AMPK, and an increased number of mitochondria in skeletal muscles. Furthermore, HSP72 overexpression increases mitochondrial activity and restores obesity-induced insulin resistance in skeletal muscles (Chung et al., 2008). Furthermore, Liu and Brooks (2012) showed that SHS induces mitochondrial biogenesis in the skeletal muscles through the activation of the HSP72/SIRT1 pathway, along with a subsequent increase in the expression of *PGC1α* and *TFAM*, which regulates mitochondrial biogenesis. However, to the best of our knowledge, no studies have investigated whether SHS is effective for the activation of SIRT1, as well as the improvement of the developmental abilities of oocytes.

In the present study, we incubated porcine oocytes at 41.5 °C, which is 3 °C higher than the normal culture conditions, for the first 1 h at the initial time point of in vitro maturation and examined the

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https://doi.org/10.1016/j.jtherbio.2018.04.010
Received 18 December 2017; Received in revised form 21 February 2018; Accepted 22 April 2018
Available online 24 April 2018
0306-4565/

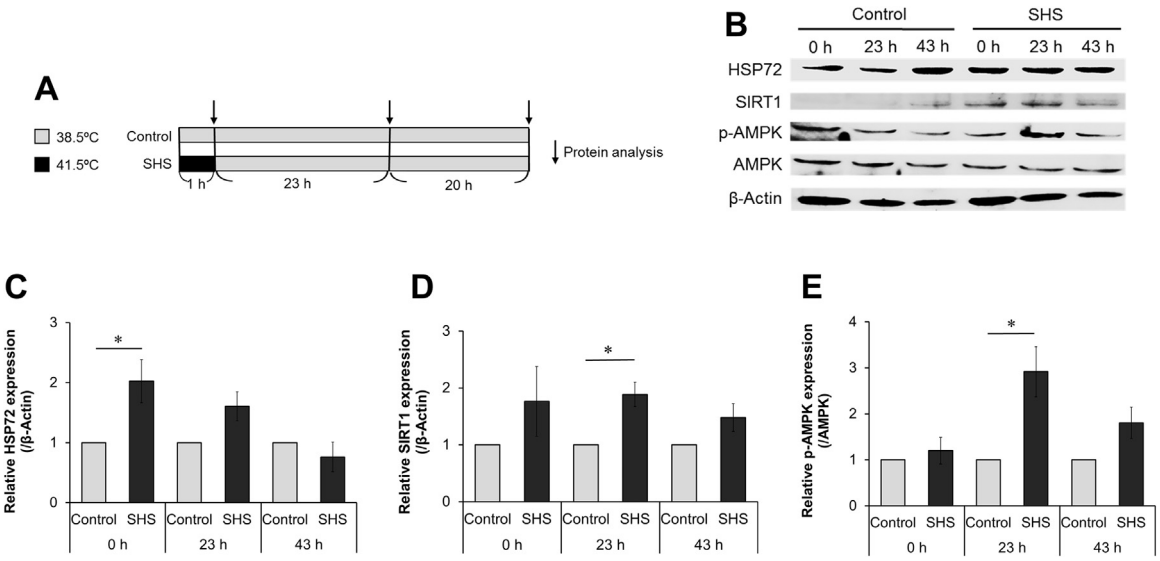

Fig. 1. Molecular response of oocytes to SHS. Experimental design of the effect of SHS on oocytes (A). Representative images of western blotting of SHS-treated and untreated oocytes (B). Protein expression of HSP72 (C), SIRT1 (D), and p-AMPK (E) in SHS-treated and untreated oocytes at 0, 23, and 43 h post-treatment.

Table 1
Primer sequences used for real-time PCR.

Gene	Primer		Accession number	Amplicon size (bp)
PGC1α	F	ttccgtatcaccacccaaat	NW_213963	137
	R	atctactgcctggggacctt		
TFAM	F	ggcagactggcaggtgta	NW_001130211	164
	R	cgaggtctttttggtttttcca		
GAPDH	F	gagtccactggtgtcttcacg	NW_001206359	236
	R	atgagtccctccacgatgc		
COX2	F	cgagagagcactttccaagg	NC_000845.1	152
(Mitochondrial DNA)	R	ctaattcgggtgttggtgct		

PGC1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1a).
TFAM: Mitochondrial transcription factor A.
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.
COX2: Cytochrome c oxidase subunit II.

mitochondrial kinetics and developmental ability of the oocytes. In addition, we investigated the potential mechanism underlying SHS-induced mitochondrial generation in oocytes.

2. Materials and methods

2.1. Chemicals and media

All chemicals used in this study were purchased from Nacalai Tesque (Kyoto, Japan) unless otherwise indicated. The medium used for in vitro maturation (IVM medium) was porcine oocyte medium (POM) supplemented with 3 mg/mL polyvinyl alcohol (Yoshioka et al., 2008), 0.5 mM L-cysteine, 10 ng/mL epidermal growth factor (Sigma-Aldrich, St. Louis, MO), 10 IU/mL equine chorionic gonadotropin (ASKA Pharma Co. Ltd, Tokyo, Japan), and 10 IU/mL human chorionic gonadotropin (Fuji Pharma Co. Ltd, Tokyo, Japan). The medium used for in vitro embryo culture (IVC medium) was porcine zygote medium 3 (PZM3, Yoshioka et al., 2002). Chemical reagents, including the proteasomal inhibitor (MG132; Wako, Osaka, Japan), and HSP72 activator (BGP-15; Sigma Aldrich), were added to the medium.

2.2. Collection of ovaries and cumulus cell oocyte complexes (COCs)

The ovaries were collected from the prepubertal gilts at a local slaughterhouse and transported to the laboratory within 1 h at 37 °C in phosphate-buffered saline (PBS) containing antibiotics. COCs measuring 3 − 6 mm in diameter were retrieved from the antral follicles by using a 21 G needle (Terumo, Tokyo, Japan) connected to a 10-mL syringe (Terumo). Oocytes with multiple compact granulosa layers and even cytoplasm were selected and pooled.

2.3. SHS treatment and in vitro maturation of oocytes

We subjected the COCs to SHS (41.5 °C) for 1 h. Control COCs were cultured at 38.5 °C. The treatment temperatures and durations were determined in a previous report (Liu and Brooks, 2012), and in our pre-experimental use of long-term heat stress, in which the nuclear maturation of oocytes was inhibited (Supplementary table). These complexes were subsequently incubated for 43 h. After incubation, the oocytes were denuded and used for experiments. IVM was performed at 38.5 °C in an atmosphere containing 5% CO_2 and 95% air.

2.4. Western blotting

After SHS, the oocytes were collected at the designated time points (Fig. 1A) and used for western blotting. Twenty oocytes were used to harvest proteins in 10 μL of cell lysis buffer (Complete Lysis-M; Roche, Basel, Switzerland) containing protease inhibitors (Complete protease inhibitor cocktail, 1 tablet/10 mL; Roche) and phosphatase inhibitors (PhosSTOP, 1 tablet/10 mL; Roche), and frozen at − 80 °C until use. Protein samples were prepared by adding Laemmli sample buffer containing 2-mercaptoethanol to each sample, followed by boiling at 95 °C