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Heat stress has no effects on activation of MAPK proteins

Yasuharu Oishi, Naoki Oishi*, Tomonori Ogata**

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Adult male Wistar rats were subjected heat stress by immersion of their hindlimb into 42-degree of hot water 5-time every other day in the present study. Heat shock protein (Hsp) 72 was increased in the fast plantaris muscle and tend to increase in the slow soleus. Mitogen-activated protein kinases (MAPKs) proteins, ERK1/2 and p38, were unaffected in either muscles by heat stress. Our results may suggest that only the heat stress is insufficient, and other factor(s) such as muscle fiber contraction concomitant with changes of intra-/inter-cellular conditions need to activate MAPK proteins.

Key words : heat stress, ERK1/2, p38 MAPK, skeletal muscle, rat

Introduction

It is well known that heat stress up-regulates specific proteins, so-called heat-shock proteins (HSPs), in mammalian skeletal muscles, which act as a molecular chaperone to maintain protein homeostasis and protect protein degradation (Thakur et al., 2018). Most popular HSP is the Hsp72, a molecular weight of 72kDa protein, expressing highly in the slow-type anti-gravity soleus muscle and lower in the fast-type plantaris muscle in rodent skeletal muscles. We previously reported that Hsp72 is specifically expressed in slow-twitch type I, but fewer in fast-twitch type II fiber in sedentary rat skeletal muscles (Ogata et al., 2003). With heat stress, Hsp72 was gradually increased and peaked at recovery 4-h in the soleus or at recovery 2-h in the plantaris after 1-h exposure of rat hindlimbs at 42-degree of hot water (Oishi et al., 2002).

Mitogen-activated protein kinases (MAPKs) are Ser/Thr protein kinases that regulate gene expression, mitosis, metabolism, motility, apoptosis, and cell proliferation and differentiation. Conventional MAPKs comprise the extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun amino(N)-terminal kinase (JNK), p38, and ERK5 (Cargnello and Roux, 2011). Concerning the roles of ERK1/2 in mammalian skeletal muscles, some studies have reported that ERK1/2 was required for myoblast proliferation/differentiation (Jones et al., 2001; Li and Johnson, 2006) and modulation of muscle-fiber type and size (Shi et al., 2008; Shi et al., 2009). In our previous study, we observed that ERK1/2 protein expression was closely related to the fast-twitch fiber phenotype in rat skeletal muscles (Oishi et al., 2019). Many studies have also reported that p38MAPK modulated adaptation of skeletal muscles after exercise/training, and proliferation and differentiation of muscle satellite cells (Aguiar et al., 2016; Nicoll et al., 2017; Parker et al., 2017).

Recently, Fan et al., suggested the interaction between the Hsp72 and p38MAPK in regulating myoblast differentiation during regenerating process of skeletal muscle fibers (Fan et al., 2018). Liu et al., also suggested that in human placenta-derived multipotent cells, heat stress activated p38MAPK and Akt signaling, which in turn activated heat shock factor 1, then leading upregulation of heat shock proteins (Liu et al., 2022).

In the present study, heat stress was applied to rat hindlimb muscles to clarify whether the activated (i.e., phosphorylated) MAPK proteins up-regulated or not, as a first step to make sure the relationship between Hsp72 and MAPKs.

Materials and Methods

This study was followed by the Japanese Physiological Society *Guide for the Care and Use of Laboratory Animals*. Adult 8-week-old male Wistar rats (body

* Rehabilitation department, Ueki Hospital
** Faculty of Human Environmental Studies, Hiroshima-Shudo University

weight: 210~240g) were used in the present study and divided into two groups: a sedentary Control (Con, n=8) and Heat stress (Heat, n=9). Heat stress was applied 5-time to the Heat rats by immersion their hindlimb into 42-degree of hot water for 30min every other day, and the soleus and plantaris muscles of both groups were excised 24-hour after the final application of the heat stress. The muscles were weighted, homogenized, and centrifuged at 12,000g for 20min. The supernatants were used for protein analyses for Hsp72, ERK1/2, and p38MAPK. The details of the procedures for SDS-PAGE and Western blotting were described elsewhere (Oishi et al., 2009).

All data were presented means and SEM, and significant differences between the groups were determined by Student-t test. Statistical significance was set at $p<0.05$.

Results

Body and muscle weights. The body weight of the Heat rats was 4.9% lower, but not significant, than that of the Con rats (269g vs. 283g) (Fig. 1). The soleus muscle weight was not different between the Con and Heat groups (106mg vs. 110mg), whereas the plantaris weight was 6.0% lower in the Heat rats (265mg) than the Con value (282mg), but not significant (Fig. 1).

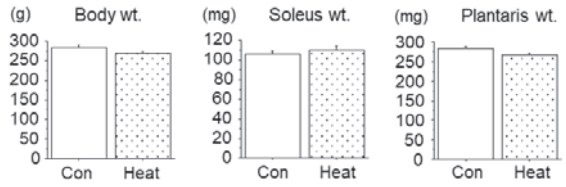

Figure 1. Body and muscle weights in Control (Con) and Heat stressed (Heat) rats.

Protein expression levels. Hsp72 protein expression level of the Heat soleus muscle tended to increase by16.2%, and that of the Heat plantaris was significantly up-regulated by 95.2%, compared with the value of the Con (Fig. 2).

The protein expression level of total p38 MAPK was similar between the Con and Heat groups of both the soleus and plantaris muscles (Fig.2), whereas the phosphorylated p38 MAPK protein level of the Heat rats tended to decrease in the soleus by 11.3% or in the plantaris by 7.1%, but neither significant, compared with the Con values (Fig. 2).

No difference was observed in the total ERK1/2 MAPK protein between the Con and Heat groups of either the soleus or plantaris muscle (Fig. 2). Also the phosphorylated ERK1/2 MAPK protein level was unaffected by heat stress in either the soleus or plantaris muscle (Fig. 2).

Discussion

In the present study, Hsp72 protein was increased by 16.2% and 95.2% in the soleus and plantaris, respectively, in the Heat rats compared with the Con, suggesting that heat stress applied in the present study was sufficient to up-regulate the heat shock protein, although phosphorylation (i.e., activation) levels of ERK1/2 and p38 MAPK proteins were unaffected by heat stress.

In sedentary condition, Hsp72 is abundant in the slow-antigravity soleus muscle, compared with the fast plantaris muscle, depending on the percentage of slow-type I fiber including in those muscles (i.e., higher in the soles and fewer in the plantaris) (Ogata et al., 2003). A small amount increase of Hsp72 is consider to be relatively a large increased ratio (95.2%) of Hsp72 observed in the Heat plantaris, compared with the Heat soleus (16.2%), of the present study.

In skeletal muscle, some studies reported that ERK1/2 is important for the expression of slow-type I fiber phenotype (Dupont et al., 2011; Meissner et al., 2011), while in our recent study, we observed that activated (i.e., phosphorylated) ERK1/2 was closely related to the fast-type II fiber phenotype in rat hindlimb muscles (Oishi et al., 2019). No studies have been reported the effects of heat stress on the activation of ERK1/2 protein, and the present study indicated that heat stress had no effects on the expression of phosphorylated ERK1/2 protein.

Mechanical stress is known to up-regulate p38MAPK phosphorylation. Luciano et al., reported that 12-week of resistance training (4-time/week) increased ERK1/2 and p38 MAPK proteins on rat quadriceps muscle (Luciano et al., 2017). They indicated that the phosphorylation levels of the ERK and p38MAPK proteins were highest in the hypertrophy-resistance training than other endurance- or strength-resistance training, suggesting the training style (i.e., intensity and/or volume) is important factor to up-regulate those proteins. In human studies, Combes et al., reported that intermittent cycling exercise up-regulated phosphorylated p38MAPK protein by 4.2-fold in human

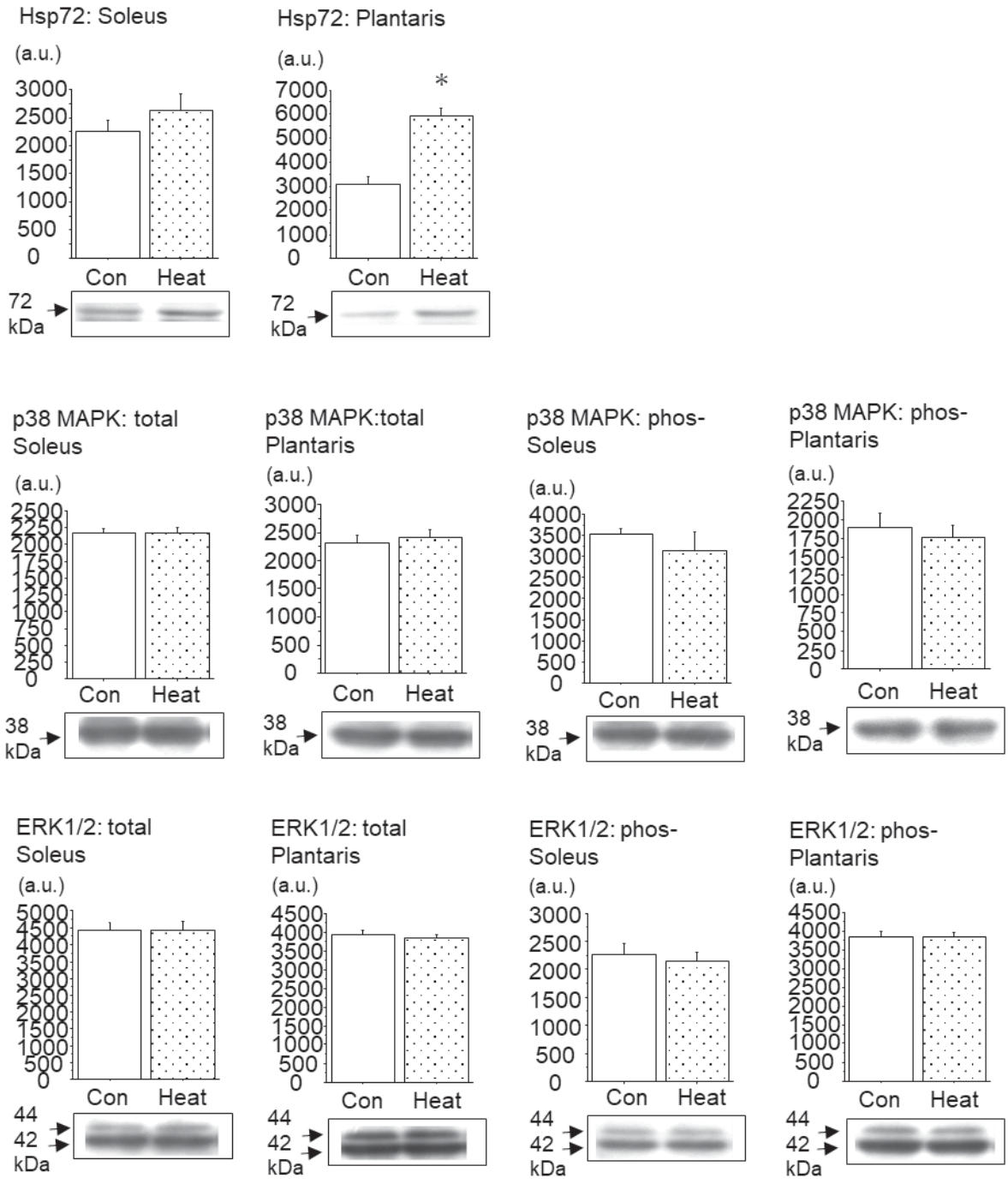

Figure 2. Protein expression level in Control (Con) and Heat stressed (Heat) rats. Protein expression pattern analysed by Western blotting was indicated under the each bar.*, $p<0.05$: phos-, phosphorylated: a.u., arbitrary unit

vastus lateralis muscle (Combes et al., 2015). Also, Yu et al., observed the increase of phosphorylated ERK 1/2 and p38 MAPK proteins by 7.8- and 4.4-fold, respectively, in human vastus lateralis after marathon running (Yu et al., 2001). Thus, the exercise and training seem to be important factor(s) to activate MAPK signaling cascade.

Compare with the present study, mechanical stress is accompanying the fiber contraction with the elevation of muscle temperature. Only the heat stress, i.e., lacking muscle fiber contraction and related metabolic changes, may be insufficient to induce the activation of MAPK proteins. Concerning this, Widegren et al., reported that some factors, such as blood flow, hormones, energy

depletion, hypoxia, lactate accumulation, decreased pH, and muscle damages, may stimulate signal transduction and then induce muscle adaptations (Widegren et al., 2001).

In conclusion, heat stress was applied to the rat hindlimb muscle by immersion into 42-degree of hot water 5-time every other day in the present study. Heat shock protein 72 was increased in the plantaris muscle and tend to increase in the soleus, but no effects were observed on the expression levels of activated MAPK proteins. Our results may suggest that muscle fiber contraction with the changes of related intra- and inter-cellular conditions need to activate the MAPK proteins. Further investigations need to clarify the relationship between the skeletal muscle fibers and MAPK signaling.

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