BRAIN RESEARCH 1466 (2012) 128–136

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Mild hypothermia facilitates the expression of cold-inducible RNA-binding protein and heat shock protein 70.1 in mouse brain

*Tomomi Kaneko**, *Kazuhiko Kibayashi*

Department of Legal Medicine, School of Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan

ARTICLE INFO

Article history:
Accepted 1 May 2012
Available online 15 May 2012

Keywords:
Mild hypothermia
Cold ambience
CIRP
hsp70.1
Cytokine
Cell survival

ABSTRACT

An appropriate thermal control system is essential for maintaining brain homeostasis. Hypothermia is a decrease in core body temperature that occurs when the thermoregulatory responses of homeothermic animals are impaired by environmental and situational influences, such as cold ambience and anesthesia. In recent years, hypothermia has been used for medical treatment, i.e., therapeutic hypothermia, for patients with stroke, traumatic brain injury, and heart surgery. However, the target molecules acting during hypothermia have not been identified. To understand the molecular mechanisms, we generated a mouse model of mild hypothermia (1 °C–2 °C below normal), and analyzed the expression of several genes. After mice were exposed to cold for 24 and 48 h, their rectal temperature reached 33 °C–35 °C. Then, using real-time quantitative PCR, we analyzed the mRNA expression levels of *c-fos*, *cold-inducible RNA-binding protein (CIRP)*, *heat shock protein (hsp) 70.1*, *oxytocin*, and representative inflammatory cytokines, i.e., *tumor necrosis factor (TNF)-α* and *interleukin (IL)-6* in target organs. Importantly, we found that the expression levels of *CIRP* and *hsp70.1* were elevated in the olfactory bulb within 48 h. In the hypothalamus, *CIRP* expression levels increased and were followed by an increase in *hsp70.1* expression. Meanwhile, *TNF-α* and *IL-6* expression decreased gradually over 24 and 48 h in the olfactory bulb and hypothalamus. These specific expression profiles, i.e., enhanced *CIRP* and *hsp70.1* expression and depressed cytokine expression, suggest that they could regulate apoptosis related to the cytokine signaling.

1. Introduction

Thermoregulatory systems play an important role in balancing heat production and heat dissipation in response to environmental changes. Prolonged exposure to cold results in hypothermia, a condition in which the core body temperature falls below the biogenic temperature range of warm-blooded animals. In humans, hypothermia is classified according to the core body temperature: mild (33 °C–35 °C), moderate (30 °C–32 °C), severe (27 °C–29 °C), and extreme (<20 °C), and

* *Corresponding author*. Fax: +81 3 5269 7300.
E-mail address: kaneko@research.twmu.ac.jp (T. Kaneko).

Abbreviations: CIRP, cold-inducible RNA-binding protein; OXT, oxytocin; Tes, testis; Ob, olfactory bulb; Ht, hypothalamus; Kid, kidney; Fc, frontal cortex; RT, rectal temperature

0006-8993/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2012.05.001

can often cause physiological changes, side effects, and abnormal behavior, e.g., paradoxical undressing and terminal burrowing (Carter et al., 1995). Mild hypothermia has been used for the treatment of ischemic injury, stroke, and cardiac arrest (Furuse et al., 2007; Mayer et al., 2004; Zeiner et al., 2000). Despite growing knowledge of hypothermia, the changes of biomolecules in the brain or other organs during hypothermia were not well understood, therefore, it is necessary to describe the patterns of gene expression in the brain that are related to thermoregulatory mechanisms. Torpor/hibernation is a similar condition to hypothermia in which the body temperature drops, and a number of gene candidates have been identified in this state. Although some of these genes could be common with hypothermia, their function and expression pattern might not be the same as during hypothermia because torpor consists of several phases, and the core body temperature drops to near ambient during the controlled state (Morin and Storey, 2009).

Recent investigations on hypothermia have indicated that several transcription factors and hormones are related to thermoregulation in cultured cells and genetically modified animals (Kasahara et al., 2007; Sakurai et al., 2006). The immediate early gene *c-fos* is an indicator of neural activity, and its expression is induced by a wide variety of stimuli (Kibayashi et al., 2009). For instance, the expression of *c-fos* is elevated in hypothalamic nuclei when animals are exposed to cold (Kasahara et al., 2007). Cold-inducible RNA-binding protein (CIRP) was also identified as a protein induced by hypothermia (Nishiyama et al., 1997). Furthermore, the expression of *heat shock protein (hsp) 70.1* mRNA is induced not only by heat stress but also by cold stress in cultured mammalian cells (Liu et al., 1994). A recent report demonstrated that *oxytocin (OXT)*-deficient mice exhibited lower body temperatures than wild-type animals when they were exposed to cold (Kasahara et al., 2007). In addition, some cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 are well-known endogenous pyrogens and inflammatory cytokines that can be induced by different forms of stress, and their expression patterns may be changed by stress and stimulation from the environment. However, the expression of TNF-α and IL-6 decreased in the ischemic rat brain following hypothermia treatment, while their expression was induced in cultured monocytes under mild hypothermia (Matsui et al., 2006; Shintani et al., 2011). These results suggest that cytokines modulate the inflammatory reaction at the site of brain injury, but it remains unknown whether this is a general effect or a hypothermia-specific effect.

To characterize gene expression profiles during hypothermia, we produced a mouse model for mild hypothermia by exposing these animals to cold stress. We hypothesized that gene expression levels would change during hypothermia. We focused on the mRNA expression levels of *c-fos*, *CIRP*, *hsp70.1*, *OXT*, *TNF-α*, and *IL-6* using real-time quantitative PCR in target areas, i.e., the olfactory bulb, hypothalamus, testis, and kidney. Furthermore, we compared the gene expression levels between brain tissues and other regions during mild hypothermia, and found that the expression levels of *CIRP* and *hsp70.1* increased, while *TNF-α* and *IL-6* expression decreased.

2. Results

2.1. Behavior of mice in the cold

To prepare hypothermic mice, the animals were exposed to cold (4 °C–5 °C) for 24 or 48 h. Rectal temperature was measured in hypothermic and normothermic mice (23 °C for 24 h, N=20; 4 °C for 24 h, N=20; 4 °C for 48 h, N=12). The rectal temperature was 36 °C–37 °C in the normothermic mice (Fig. 1). Conversely, after exposure to cold, the rectal temperature began to drop after ~3 h; 24 h later, it reached 33 °C–35 °C (Fig. 1). As reported previously, body weight and ingestion of food tend to increase during cold acclimation (Lapo et al., 2003; Nishimura et al., 1999). We also found that the volume of egestion increased after the experiment, even after exposure to cold for a short period (data not shown). Generally, the eccrine sweat glands, which are controlled by the sympathetic nervous system, regulate body temperature. In rodents, the glandular system is immature, and found only on the digits and footpads of the paws (Sato et al., 1994). The increase in egestion suggests that normal sweating from these glands was decreased in cold temperatures. When male mice from different cages are put together, they usually fight each other. Surprisingly, we observed that most mice huddled together in the cold. However, a small number of mice continued to move about the cage, but all cold-exposed mice finally showed shivering behavior and huddled together with the other mice. This suggests that the mice changed their normal behavior to survive the colder conditions.

2.2. Mild hypothermia can activate or repress several genes in the target tissues

To elucidate the molecular mechanisms of hypothermia, we analyzed the expression of several genes in our mouse model

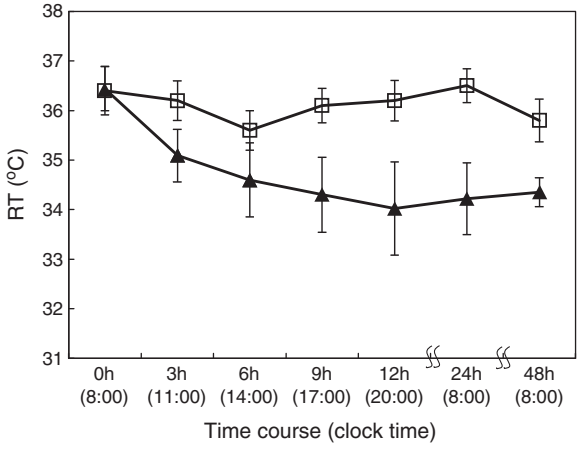

Fig. 1 – Rectal temperature of the mice. Rectal temperature was measured in hypothermic (black triangles) and normothermic (white squares) mice. After 24 h, the rectal temperature of the mice in cold chamber reached 33 °C–35 °C. At 4 °C: 0 h, N=20; 3 h, N=16; 6 h, N=18; 9 h, N=19; 12 h, N=15; 24 h, N=20; and 48 h, N=12. At 23 °C: 0 h, N=12; 3 h, N=10; 6 h, N=10; 9 h, N=10; 12 h, N=9; 24 h, N=20; and 48 h, N=6. RT = rectal temperature.