Body temperature regulation during acclimation to cold and hypoxia in rats

V. Cadena *, G.J. Tattersall

Department of Biological Sciences, Brock University, St. Catharines, ON, Canada L2S 3A1

ARTICLE INFO

ABSTRACT

Extreme environmental conditions present challenges for thermoregulation in homoeothermic organisms such as mammals. Such challenges are exacerbated when two stressors are experienced simultaneously and each stimulus evokes opposing physiological responses. This is the case of cold, which induces an increase in thermogenesis, and hypoxia, which suppresses metabolism conserving oxygen and preventing hypoxaemia. As an initial approach to understanding the thermoregulatory responses to cold and hypoxia in a small mammal, we explored the effects of acclimation to these two stressors on the body temperature (T_b) and the daily and ultradian T_b variations of Sprague-Dawley rats. As T_b is influenced by sleep-wake cycles, these T_b variations reflect underlying adjustments in set-point and thermosensitivity. The T_b of rats decreased precipitously during initial hypoxic exposure which was more pronounced in cold ($T_b = 33.4 \pm 0.13$) than in room temperature ($T_b = 35.74 \pm 0.17$) conditions. This decline was followed by an increase in T_b stabilising at a new level $\sim 0.5\,°C$ and $\sim 1.4\,°C$ below normoxic values at room and cold temperatures, respectively. Daily T_b variations were blunted during hypoxia with a greater effect in the cold. Ultradian T_b variations exhibited daily rhythmicity that disappeared under hypoxia, independent of ambient temperature. The adjustments in T_b during hypoxia and/or cold are in agreement with the hypothesis that an initial decrease in the T_b set-point is followed by its partial re-establishment with chronic hypoxia. This rebound of the T_b set-point might reflect cellular adjustments that would allow animals to better deal with low oxygen conditions, diminishing the drive for a lower T_b set-point. Cold and hypoxia are characteristic of high altitude environments. Understanding how mammals cope with changes in oxygen and temperature will shed light into their ability to colonize new environments along altitudinal clines and increase our understanding of how T_b is regulated under stimuli that impose contrasting physiological constraints.

1. Introduction

Much of a mammal's energy is devoted to the regulation of body temperature (T_b). Indeed, homoeothermy, which is the maintenance of relative constancy in T_b, is a characteristic feature of mammalian physiology, and heat generation through metabolism is the main thermoregulatory mechanism of endotherms. The regulation of T_b in mammals is centrally controlled by the preoptic area of the anterior hypothalamus (POAH). This hypothalamic model for temperature regulation proposes that thermal and other environmental information is conveyed from the periphery to the hypothalamus where it is integrated, and thermoeffectors activated to achieve or maintain a certain T_b (Hammel et al., 1963; Heller et al., 1978; Boulant, 1981). Thermal motor outputs (i.e. thermoeffectors) such as behavioural mechanisms of thermoregulation, peripheral vasomotion (which modulates the amount of heat lost through the periphery) and metabolic heat production are, therefore, coordinated by the hypothalamus to correct any deviations in temperature in both a feedback and feedforward manner (Hammel et al., 1963; Heller et al., 1978; Boulant, 1981; Kanosue et al., 2010).

Extreme environmental conditions such as cold or hypoxia (low oxygen concentration) present challenges to homoeothermy and affect the way T_b is controlled and regulated (e.g. Tattersall and Milsom, 2009). Depending on the degree and length of cold exposure as well as on the species, T_b may be maintained or hypothermia may ensue. For example, the T_b of Wistar rats exposed

to 5 °C decreases by ∼1 °C during the first hour of exposure to cold (Gautier et al., 1991) while the T_b of golden hamsters did not change significantly at ambient temperatures between 30 and −15 °C but dropped by 7 °C when exposed to −30 °C (Pohl, 1965). Prolonged cold exposure induces biochemical and morphological changes that aid in maintaining T_b at a normothermic level (Gordon, 1993b). The main physiological adjustment during cold acclimation in small mammals is a shift from shivering to non-shivering thermogenesis (Hart et al., 1956). This is mainly due to molecular remodelling (*e.g.* up-regulation of uncoupling protein 1; UCP1) and an increase in the size of brown adipose tissue (Himms-Hagen, 1990; Cannon and Nedergaard, 2004; Beaudry and McClelland, 2010), a heat generating tissue, highly vascularized and rich in mitochondria (Gordon, 1993a). As a result of these changes in BAT, the basal metabolic rate (BMR) of most small mammals increases with acclimation to cold (Gordon, 1993c), since a constitutive component of thermogenesis remains activated even when the rat is within the usual thermo-neutral zone.

The metabolic response to hypoxia is opposite to that of cold. During initial hypoxic exposure, oxygen consumption decreases (Mortola, 1993), thereby protecting organs from tissue damage due to oxygen deficit (Wood, 1995). Along with metabolic suppression, the T_b of mammals is regulated at a lower level (*i.e.* the set-point for T_b is lowered) and this is achieved through the coordination of different autonomic and behavioural thermoeffectors that facilitate heat loss and, as previously mentioned, decrease thermogenesis through a decrease in metabolism (*e.g.* Mortola and Feher, 1998; Tattersall and Milsom, 2003). Furthermore, Tattersall and Milsom (2009) showed that the activation of thermogenic mechanisms is effected by acute hypoxia through a decrease in the thermal sensitivity of the hypothalamus, at least for the activation of cold thermoeffectors (Hammel et al., 1963; Morrison et al., 2008).

The T_b of Sprague Dawley rats and CD-1 mice slowly increases after the drop that occurs during the initial exposure to hypoxia (Bishop et al., 2000; Mortola and Seifert, 2000; Beaudry and McClelland, 2010). After approximately one day, the T_b of the rats reaches pre-hypoxic levels, albeit with blunted circadian fluctuations which remain considerably diminished even after two weeks of continuous hypoxia (Bishop et al., 2000; Mortola and Seifert, 2000). By the fourth week of acclimation to hypoxia, CD-1 mice exhibit T_b values and circadian variations similar to those of normoxic control mice (Beaudry and McClelland, 2010) although it is not clear whether blunting of the daily T_b oscillations occurs in the earlier stages of hypoxic exposure in this species.

Because of the contrasting effects of cold and hypoxia on thermogenesis and T_b, environments such as high altitude in which both stressors are present introduce challenges to the way in which homoeothermy is achieved. To our knowledge, only one study has addressed the effects of acclimation to both cold and hypoxia on the thermoregulation of a mammal (Beaudry and McClelland, 2010). This study showed that after four weeks of cold and hypoxia acclimation the T_b of mice was up to ∼4 °C lower than the T_b of mice acclimated to only one of these stimuli. Hypoxia and cold acclimated mice also lacked the circadian oscillations observed in cold acclimated, hypoxia acclimated or control mice (Beaudry and McClelland, 2010). Furthermore, several physiological and behavioural processes have been shown to exhibit ultradian rhythmicity (*i.e.* periodic cycles shorter than 24 h) in the rat. Some of these include metabolism, heart rate, arterial pressure, feeding, and brain and body temperatures (Shimada and Marsh, 1979; Alfoldi et al., 1990; Holsteinrathlou et al., 1995). These parameters increase in an integrated manner with arousal state during sleep cycles as well as with rest-activity cycles during wakefulness (Roussel and Bittel, 1979; Obal et al., 1985; Blessing, 2012). Given that thermosensitivity is reduced during acute

hypoxia (Tattersall and Milsom, 2003; Scott et al., 2008; Tattersall and Milsom, 2009) and that daily T_b cycles are blunted due to inhibition of thermogenesis after prolonged (more than 24 h) exposure to hypoxia it is possible that other cyclic variations in T_b, such as ultradian variations, are also affected by hypoxia. A decrease in T_b ultradian variations, and therefore, the diminishment in the magnitude and frequency of periodic increases in thermogenesis during hypoxia would be consistent with the hypothesis that a suppression in metabolic rate is beneficial under low oxygen conditions. However, the effects of hypoxia, or of cold and hypoxia combined, on ultradian rhythms of body temperature have not yet been examined.

As an initial approach to understanding the thermoregulatory responses to cold and hypoxia in a small mammal, we explored the effects of acclimation to these two stressors on the T_b of Sprague-Dawley rats. This study examined whether the set-point for temperature regulation is flexible during exposure to hypoxia and is adjusted according to other environmental factors, such as ambient temperature. We also wanted to examine the effect of hypoxia on daily T_b variations and ultradian T_b rhythms; ultradian rhythms of body temperature have been shown to exhibit circadian rhythmicity (Gordon, 2009) and are the result of activity, sleep–wake cycles and feeding bouts (Roussel and Bittel, 1979; Obal et al., 1985; Blessing, 2012). Given that hypoxia induces a depression of activity and body temperature circadian rhythms, and decreases food consumption (Bishop et al., 2000; Mortola and Seifert, 2000; Beaudry and McClelland, 2010), hypoxia, independent of ambient temperature should induce a decrease in the daily rhythmicity of ultradian body temperature variations. A decrease in periodic (both daily and ultradian T_b rhythms) activation of metabolic thermogenesis would support the hypothesis that the thermoregulatory changes during hypoxia are coordinated towards minimizing oxygen consumption and preventing oxygen deficit in vital organs and tissues. Lastly, we hypothesized that the set-point for temperature regulation is adjusted throughout the course of acclimation to hypoxia as the animal's physiological functions adjust to increase oxygen uptake and delivery. To explore these questions we examined and compared the time course of the cold, hypoxic and cold/hypoxic responses on different aspects of T_b such as changes in mean T_b and variations in ultradian rhythms and daily T_b variations. Understanding how body temperature changes during acclimation to cold and hypoxia will not only help us understand how small mammals adjust to high altitude environments but will also shed light on how thermal homoeostasis is maintained under conflicting physiological stressors through adjustments in the body temperature set-point.

2. Materials and methods

2.1. Animals

Data for this study was obtained from a total of 115 male Sprague-Dawley rats (Charles Rivers Laboratories Inc., St. Constant, QC, Canada) from other studies that explored different aspects of temperature regulation during acclimation to cold and/or hypoxia (unpublished data). All animals were provided with laboratory rat chow and water *ad libitum* and housed in a facility at Brock University maintained at a 12:12 h light–dark condition. Depending on the study of origin of the rat, lights were on at 08:00 h or 20:00 h. In cases in which the photophase was reversed (*i.e.* lights on at 20:00 h), rats were allowed a minimum of two weeks of adjustment before any data were obtained. All aspects of this study were performed according to the Canadian Council of Animal Care (CCAC) guidelines and approved by the Brock University Animal Care and Use Committee (ACUC).