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Sex Differences in Stress-Induced Hyperthermia in Rats: Restraint Versus Confinement

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Abstract

Studies using restraint to induce psychological stress consistently report the expected hyperthermic response in core body temperature (CBT), but many also report a hypothermic response that precedes the hyperthermia. To understand the conditions that produce hypothermia, and to study sex differences in stress-induced hyperthermia, we measured CBT in male and female rats at 70 and 180 days of age in response to two types of stressors: immobilization through restraint (Plexiglas restrainer) and confinement in a small area (circular wire mesh cylinders that allowed free airflow). Restraint early in the light period induced hypothermia only in 180-day-old males, with no hyperthermia observed during the 30-minute restraint period. Increases in humidity and temperature of the microenvironment due to the larger body weight at this age may contribute to the hypothermia. Hyperthermia during restraint in 70-day-old males was significantly attenuated and delayed in onset compared to the rise in females. All females exhibited a CBT rise of approximately 1.3°C occurring 10–15 minutes after the onset of restraint. Restraint early in the dark period induced no significant change in CBT in males of either age during immobilization, while females exhibited a small rise of approximately 0.5°C. Confinement early in the light period induced a significant rise of approximately 1.5°C in all groups, with no preceding hypothermia. However, the male response was significantly delayed compared to females. Overall, these results indicate that CBT changes during restraint likely involve both anxiogenic and physiological components, while the marked hyperthermia during confinement is primarily psychological in both sexes.

Keywords

Restraint; core body temperature; sex differences; stress; thermoregulation

INTRODUCTION

Activation of the sympathetic nervous system in response to perceived threat, or generalized psychological arousal, causes an increase in core body temperature (CBT) in animals and humans that is similar in both warm and cold environments and not related to increased locomotor activity (Diorio et al.; 1993; Hansel and Kanel, 2008; Takakazu et al., 2001). The

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magnitude of this psychogenic response is generally limited by the normal upper limit of body temperature. Hyperthermia in humans has been observed following overt and suppressed anger, anticipation of the start of a boxing match, or test taking (Briese, 1995; Marazziti et al., 1992). Similar rises are observed in rodents following exposure to a novel situation, social defeat, saline injection, handling, exposure to noise, anticipation of threat, or restraint (Barnum et al., 2007; Olivier et al., 2008).

Many animal models of psychological stress employ restraint as a method to induce hyperthermia because of the control this paradigm provides over the timing and duration of the stress, as well as the reliability of the response (Bouwknicht et al., 2007; Vinkers et al., 2008). However, interpretation of stress-induced hyperthermia in restrained rodents can be complicated by a hypothermic component that precedes the hyperthermic stress response in some studies (e.g., Chen and Herbert, 1995a,b,c; Chung et al., 2000; Johnson et al., 2000, 2004; Stamp and Herbert, 1999, 2001; van Eijl et al., 2006), but not others (e.g., Hashimoto et al., 2001; Sanches et al., 2002, 2003; Terlouw et al., 1996; Vidal et al., 1984; Wright and Katovich, 1996). Investigators observing this hypothermic component have generally considered it part of the anxiogenic response (e.g., Chung et al., 2000; Johnson et al., 2004; Stamp and Herbert, 2001).

When hypothermia is observed in studies using restraint, the procedure generally involves use of plastic restraint tubes that keep the animal in a prone position. Furthermore, males are the only sex that has been tested for this response. Although restraint tubes are usually slotted to allow some amount of aeration, effective heat dissipation is related to the size of the animal and the degree of immobility. Not all studies of CBT that use plastic restrainers report hypothermia, but this phenomenon has never been reported when restrainers were constructed from a wire mesh (Hashimoto et al., 2001; Wright and Katovich, 1996; Vidal et al., 1984).

The present experiments were designed to examine the role of plastic restrainers, weight, gender, and time of day as potential mediating variables in stress-induced hypothermia. Restraint studies reporting hypothermia have only used male animals and the potential for a sex difference in this stress response might be expected based on the higher basal core temperature found in most female mammals, including rats (Gordon, 1990). In addition, compared to males, significantly greater hyperthermic responses in females have been reported when animals are rehoused with new cage mates (Thompson et al., 2003).

Body weight was also a consideration in the design because larger animals might have more trouble dissipating heat in a plastic tube of a fixed size than smaller ones. Therefore, initial hypothermia in restrained animals might reflect a physiological response to temperature changes in the microenvironment of the tube, rather than the assumed response to anxiety. Finally, we also considered the influence of diurnal fluctuation in CBT as a potential variable, since CBT is significantly lower in the early hours of the light cycle in the rat compared to the corresponding phase of the dark cycle. To address these possibilities, we restrained male and female rats at 70 or 180 days of age for 30 minutes in standard Plexiglas restraint tubes that immobilize animals in a prone position. The hyperthermic stress response was measured one hour after the onset of the light and dark phases of the light cycle. For comparison purposes, similar groups of animals were confined for 30 minutes after the onset of the light phase of the light cycle in screen cylinders that confined the animal, but allowed free air flow and animal movement.

MATERIALS & METHODS

Subjects

Animals used in these experiments were Sprague-Dawley rats (Harlan Labs, San Diego, CA). Prior to surgery, all animals were group housed in a temperature-controlled vivarium under a 12:12 light-dark cycle (lights on at 0600). The controlled ambient temperature in the testing room was $21(\pm 1.5)$ °C. Following surgery, animals were singly housed under the same environmental conditions in a separate room designed to accommodate electronic monitoring of temperature. Animals of both sexes were either age matched (70 and 180 days of age) or weight matched (180 d females vs 70 d males). The average weight ($\pm$ standard error of the mean (SEM)) of the groups at the start of the experiment was as follows: Females 70 Days, 274g (± 9.3); Males 70 Days, 460g (± 13.7); Females 180 Days, 473g (± 17.2); Males 180 Days, 767g (± 31.4). Six animals were included for study at each age. All procedures were approved by the Animal Care and Use Committee at San Diego State University.

Transmitter Implantation and Data Collection

At least 10 days prior to the beginning of the experiment, animals were implanted with a temperature-sensing transmitter according to the manufacturer's protocol (Minimitter Co., Bend, OR). Surgeries were performed using ketamine/xylazine anesthesia (100/15 mg/kg). Following surgery, the animals were singly housed in shoebox type cages placed on receivers equipped to monitor the transmitter signals. Receivers were connected to ER-4000 data ports and information was transferred to a computer equipped with the Vital View data collection system (Minimitter Co., Bend, OR). CBT was recorded every 5 minutes.

Stress Procedure

Two different procedures were used to induce psychological stress, restraint in a clear plastic half-cylinder, and confinement within a circular screen cylinder. For restraint, we used two sizes of Plexiglas rodent restrainers (Stoelting, Wood Dale, IL), which restrained the animals in a prone position. The restrainers have slots in the walls that allow heat dissipation, depending upon the tightness of fit. The back wall of the restrainer can be moved to accommodate different lengths of animals in order to keep them immobilized, with the tail extending out beyond the wall. Males and 180 day-old females were restrained in the largest model available (model #51334; range 250–500 g), and 70 day-old-females in the smaller version (model #51333; range 125–250 g). Dimensions for the large model are 3.25" diameter $\times$ 8" in length, and 2.5" diameter $\times$ 6" in length for the smaller.

Animals were restrained in Plexiglas tubes for 30 minutes beginning at either 0700 hrs or 1900 hrs. At the end of the 30-minute stress period, the animal was returned to the home cage, and monitoring continued for another 90 minutes. Stress exposure was conducted in a counterbalanced order, with 72 hours separating the morning and evening sessions.

For confinement stress, we constructed two different size arenas (10" high $\times$ 6" diam; 8" high $\times$ 4" diameter) comprised of cylindrical walls made of galvanized screen (0.125" opening), allowing free airflow through the cylinder as well as room for the animals to make postural adjustments. Lids were made of the same galvanized screen material, while bases were constructed of epoxy-painted wood. A separate cohort of animals was used for confinement stress. These animals were tested at 0700 hrs in the large and small confinement cylinders. Half of each group was confined in the small cylinder and the other half in the large cylinder.

Each 30-minute stress exposure occurred in the room where the animals were housed, with the restraint tube or confinement cylinder placed over the home cage receiver. During the experimental period, core body temperature was sampled every 5 minutes beginning 60

minutes prior to stress onset and continuing for 90 minutes after the animals were released from confinement into the home cage.

RESULTS

Restraint Stress

Responsiveness during the 30-minute restraint stress period was calculated by subtracting each post-stress measurement (5 minute intervals) from the 15-minute average prior to stress onset. These difference scores were first analyzed using a 2 (age) $\times$ 2 (sex) $\times$ 2 (Time: AM/PM) $\times$ 6 (5 minute blocks) ANOVA, with repeated measures over the last two factors. Separate follow-up ANOVAs, with repeated measures and orthogonal trend analyses, were conducted for each age group for the AM and PM stress periods.

The initial analysis of CBT yielded main effects for Sex ($F[1,20]=68.69$, $p<0.0001$), AM/PM ($F[1,20]=34.22$, $p<0.0001$) and Block ($F[5,100]=14.85$, $p<0.0001$). Significant interactions included the following: AM/PM $\times$ Sex ($F[1,20]=25.28$, $p<0.0001$); AM/PM $\times$ Sex $\times$ Age ($F[1,20]=7.38$, $p<0.2$); Block $\times$ Sex ($F[5,20]=10.62$, $p<0.0001$); Block $\times$ AM/PM ($F[5,100]=5.17$, $p<0.001$); and Block $\times$ AM/PM $\times$ Sex ($F[5,100]=5.42$, $p<0.001$).

In the AM stress period, males at both ages exhibited a decline in CBT after the onset of stress that was not present in females (see Figure 1). However, the decline in 6 month old males was significantly greater and extended compared to their 70 day old counterparts (quadratic component ($F[1,10]=6.76$, $p<0.05$). Females at 70 days and 6 months of age responded to the stress with a maximal rise in CBT of 1.42 and 1.57 degrees C, respectively. Males at 70 days of age responded to the stress with a maximal rise of 0.43 degrees C during the stress period. Although, there was an average drop in CBT during the first 10 minutes in this group, this was not significant. In comparison with their 70 day old counterparts, 6 month- old males exhibited a significant hypothermia throughout the stress period ($F[1,10]=6.83$, $p<0.05$). Maximal hypothermia reached -0.6 degrees C at 15 minutes, and was still -0.03 degrees C below baseline when the stress period ended.

As shown in Figure 2, females also exhibited a significant rise in CBT during the PM stress period following the onset of restraint stress, whereas no significant change was observed in males during the stress period ($F[1,20]=13.97$, $p<0.01$). The maximal rise in females was 0.87 and 0.62 degrees C for 70 day old and 6 month old animals, respectively. This difference was not significant.

Confinement stress induced a marked hyperthermia in the AM stress period in both males and females, with the degree of hyperthermia significantly greater in females ($F[1,20]=16.72$, $p<0.001$). As shown in Figure 3, the onset of hyperthermia was slower in males ($F[5,20]=9.09$, $p<0.0001$). However, the slope of the rise between 15 and 25 minutes after stress onset did not differ significantly by age or sex. The maximal change in CBT during the stress period was 1.77 and 1.89 degrees C for 70 day old and 6 month old females, respectively. For 70-day-old males and 6-month-old males, it was 1.59 and 1.30 degrees C, respectively. Post hoc analyses using t-tests showed significant differences in males at both ages between the maximal rise in CBT during restraint stress versus confinement stress ($p<0.001$), but no significant difference in females between stress conditions. Data are shown in Table 1.

DISCUSSION

Results of these studies indicate that restraint-induced hypothermia may represent a physiological rather than a psychological response. We find that its appearance prior to the normal hyperthermic response depends upon several factors, including the degree of

immobilization, time of day, and perhaps the sex of the animal. Significant hypothermia preceded the normal hyperthermic response only in 6-month-old males stressed at 7AM, when CBT is near its nadir in the normal circadian fluctuation of CBT, but not at 7PM when CBT is near its peak. The hypothermia lasted throughout the 30-minute stress period and was followed by a hyperthermic response after the animals were returned to their home cage. Among the four groups of animals tested in restrainers, these were the only ones whose weight exceeded the recommendations of the manufacturer for the tubes we employed. As a result, for these large, 6 month-old males, the microenvironment was hotter and more humid relative to what the other groups experienced. This condition creates an overheated microenvironment that alters both peripheral and core thermoregulation (Wright and Katovich, 1996). Alternatively, age may have contributed to the restraint induced hypothermia in males via changes in psychological stress responsivity that normally occurs with aging (Segar et al., 2009). However hypothermia was not observed after confinement in 6-month old male rats, again indicating a potential physiological response. It also remains possible that a greater psychological response may have been elicited in 6-month old male rats because their degree of confinement was greatest during restraint, and as a result we see an altered CBT response. Therefore, further studies are needed to tease out the psychological vs. physiological contributions to the CBT response to stress.

In the literature, the hypothermic response in restrained animals is not ubiquitous and when it is observed, restraint is accomplished with Plexiglas restrainers similar to what we have used, or with plastic conical tubes. In addition, these studies used male rats and were conducted during the light phase of the light/dark cycle when CBT is at its nadir (Chen and Herbert, 1995a,b; Johnson et al., 2004; Stamp and Herbert, 1999, 2001; van Eijl et al., 2006). No studies that used a mesh restraint tube reported hypothermia (Hashimoto et al., 2001; Wright and Katovich, 1996; Vidal et al., 1984).

Restraint-induced hypothermia was first observed in physiological studies of thermoregulation conducted under changing ambient temperatures. Over time, the cause has been attributed to anxiety, increased peripheral vasodilatation, ambient temperature, or a combination of these factors (Heroux and Hart, 1954; Wright and Katovich, 1996; Johnson et al., 2000). However, if the hypothermic aspect of the stress response reflects an anxiety response as proposed, there should be a greater drop in temperature during the 7PM stress compared to the 7AM stress. At this time, basal CBT is nearly one degree centigrade higher than at 7AM, thereby providing a greater range for expression of a hypothermic response. The absence of a significant drop at 7PM suggests that the hypothermia we observed at 7AM is not directly linked to the psychological aspects of restraint, but may instead reflect circadian differences in cardiovascular tone and heat production. The higher basal temperature in the evening, combined with normal increases in heart rate and blood pressure, may have offset heat loss through heat dissipation caused by close contact with the plastic restrainer wall. The interaction between heat loss and temperature in lowering CBT is demonstrated in the studies of Heroux and Hart (1954). They observed a hypothermic response of more than 5 degrees centigrade in animals restrained at a room temperature of 30°C, but no hypothermia when the animals were restrained in a 6°C environment to which they were previously acclimatized. Interestingly, in a study of males implanted with a corticosterone pellet, restraint-induced hypothermia was significantly attenuated compared to sham operated and adrenalectomized controls (Stamp and Herbert, 2001).

The greater hyperthermic response in females to both restraint and confinement stress is consistent with their overall endocrine, autonomic and behavioral stress responsiveness (Atkinson and Waddell, 1997; Handa et al., 1994). It also corresponds to the pattern observed by Thompson et al. (2003), whereby females exhibited a greater hyperthermic response than males to re-housing with new cage mates. The hyperthermia following both restraint and

confinement stress in females of both ages exhibited a rapid rise beginning 10–15 minutes after stress onset. In contrast, the onset in males was markedly later, or absent during the stress period. In both 70-day-old and 6 month-old immobilized males, the onset of hyperthermia at 7 AM exhibited a significant lag compared to females of both ages. These observations also raise the possibility that the observed hypothermic and hyperthermic responses may represent a continuum of responses to varying the severity of the stressor. Similar patterns have also been observed in other physiological systems such as the rat model of systemic inflammation induced by bacterial lipopolysaccharide (LPS) where the pattern of temperature change is dependent on LPS dose and on ambient temperature. For example, hyperthermia is a typical response to low doses of LPS given in a neutral or warm environment, whereas hypothermia, followed by fever is the predominant response in a cool environment (Romanovsky et al., 1996, 2005).

It is also important to note that restrainer size does not seem to play a role in the observed differences. 70-day-old males, and 6-month-old females were approximately the same size and stressed in the same size restrainers, but males still exhibited a marked delay in hyperthermia. The data from confined animals at 7AM also shows a significant lag in the onset of hyperthermia in males at both ages. However, the onset of hyperthermia in confined males is much earlier than we observed under immobilization stress. It is also important to note that, to our knowledge, restraint studies of the CBT response during different stages of the estrus have not been published. Because CBT fluctuates during the estrous cycle, future studies will be needed to determine to what degree these fluctuations influence the response to restraint stress and contribute to the sex differences we observed.

Anxiogenic responses, including hyperthermia, arise from perception, which originates in the brain. In contrast, thermogenic responses arise from a whole body feedback system that involves heat loss dissipation, vasodilatation or constriction, and/or shivering (Wright and Katovich, 1996). Traditionally, hyperthermia has been thought to arise from a reflex common to both anxiogenic and thermogenic stimuli, which has been supported by measurements of brain temperature that show a strong correlation with peripheral blood temperature (Gordon, 1990). However, more recent telemetric methods that allow finer discriminations of hyperthermic response times show that anxiogenic stimuli induce a rise in brain temperature that precedes the rise in arterial blood (Kiyatkin et al., 2002; 2007). This suggests that stress-induced hypothermia involves a distinct neural pathway from the hyperthermic response induced by emotional reactivity.

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A

B

Figure 1.

(A) CBT response to 30 minutes of restraint in Plexiglas restrainers at 7AM. The stress period is designated by the black bar on the X-axis. (B) The change in temperature from baseline during the 30-minute restraint period. Data shown are mean and SEM of 6 animals/group.

Figure 2.

(A) CBT response to 30 minutes of restraint in Plexiglas restrainers beginning at 7PM. The stress period is designated by the black bar on the X-axis. (B) The change in temperature from baseline during the 30-minute restraint period. Data shown are mean and SEM of 6

Figure 3.

(A) CBT response to 30 minutes of confinement in a wire mesh vertical cylinder beginning at 7AM. The stress period is designated by the black bar on the X-axis. (B) The change in temperature from baseline during the 30-minute confinement period. Data shown are mean and SEM of 6 animals/group.

Table 1

Maximal CBT during the 7AM, 30-minute stress period. Data are the mean ($\pm$ SEM) change in degrees centigrade from the 15 minutes baseline immediately preceding stress onset.

	Males 70 days	Females 70 days	Males 6- Months	Females 6- Months
7AM Restraint	0.43 [*] (0.07)	1.42 (0.33)	-0.03 [*] (0.17)	1.56 (0.27)
7AM Confinement	1.59 (0.09)	1.78 (0.07)	1.30 [#] (0.11)	1.89 (0.19)

*
p<0.01 from females of same age.

[#]
p<0.05 from females of same age.