Heat Balance during Physical Restraint in Rats

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Abstract By using a gradient layer calorimeter built in our laboratory, heat balance during physical restraint was measured in rats at a calorimeter temperature of 25°C. After restraint, the rate of heat production ($\dot{M}$) increased sharply and significantly (from $53.86 \text{ W} \cdot \text{m}^{-2} \pm 3.12 \text{ SE}$ to $69.12 \text{ W} \cdot \text{m}^{-2} \pm 2.86 \text{ SE}$). The high $\dot{M}$ was maintained for the whole period of 2.5-hr restraint. The rate of heat loss ($\dot{H}_L$) increased progressively and reached a high value (from $54.02 \text{ W} \cdot \text{m}^{-2} \pm 2.78 \text{ SE}$ to $67.44 \text{ W} \cdot \text{m}^{-2} \pm 2.48 \text{ SE}$) 40 min after restraint. The high value in $\dot{H}_L$ was maintained ($67.44–68.12 \text{ W} \cdot \text{m}^{-2}$) and the rate of heat storage ($\dot{S}$), which was positive during the first 40 min, tended toward zero for the rest of 2.5-hr restraint. Colonic temperature ($T_{col}$), which was increased from $37.40°C \pm 0.07 \text{ SE}$ to $38.03°C \pm 0.16 \text{ SE}$ during the first 40 min, increased insignificantly during the following period of restraint. These results indicated that a thermal equilibrium was reached during a prolonged restraint at a calorimeter temperature of 25°C. After release from restraint, $\dot{H}_L$ and $\dot{M}$ decreased progressively. $\dot{S}$, which was negative in the first 60 min of post-restraint period, became zero and $T_{col}$ reached a steady value of $37.78°C \pm 0.09 \text{ SE}$.

The restraint-hypermetabolism was either reduced or completely inhibited by the chemical sympathectomy (6-OHDA, 100 mg/kg i.p.) or bilateral adrenalectomy. An increased activity of the sympathetic nervous system and an increased secretion of catecholamines might be responsible for the restraint-hypermetabolism in rats.

This report has also described a direct calorimeter for measuring heat balance in unanesthetized rats. The results obtained in this study approved the simplicity of the actual operation and the usefulness of the calorimeter in studying thermoregulatory responses in small mammals.

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BARTLETT, Jr. (1959), however, observed a marked increase in oxygen consumption in the restrained guinea pigs and concluded that the hypothermia is due to an excessive heat loss from the restrained animals. Recently, McEWEN, Jr. (1975) reported a significant increase in metabolism in restrained rabbits. Since the rectal temperature was stable even in slightly cold environments, heat losses might have been increased to the extent of the increased metabolism. In other words, heat balance should have been maintained during restraint in these animals. In the studies mentioned above, however, heat balance using calorimetric methods was not measured systematically in the restrained animals. In our laboratory, we have built a direct calorimeter of the gradient layer type for small animals. Using this instrument, heat balance was measured in rats during restraint at calorimeter temperature of 25°C. Since restrained animals are commonly used in many studies on temperature regulation, the present study can provide useful information in the understanding of experimental results obtained in restrained animals.

MATERIALS AND METHODS

Description of direct calorimeter used in this experiment. Figure 1 shows the essential features of the calorimeter. The calorimeter shell (12 × 12 × 25 cm in size), made of 2 mm thick copper plates, was mounted in the center of a plexi-glass water chamber (28 × 28 × 32 cm in size). The inner walls of the shell were covered completely with an HFT-type gradient layer (HATFIELD, 1950), except for the door. The door (1/10 of the total surface of the shell) was made of 4-cm-thick plexi-glass

Fig. 1. Diagram of the gradient layer calorimeter for small animals.
to provide good thermal insulation and transparency. The room temperature was carefully controlled at 25°C to minimize possible heat flow through the door. To maintain the temperature variation of the heat sink within ±0.01°C of the set temperature, a large amount of temperature-controlled water (24 liters/min) was circulated through the plexi-glass chamber by a water pump. Room air (1.4 liters/min) was passed through the silica gel cans and was sent into the calorimeter through copper coils (4 mm in diameter and a total length of 15 m) immersed in a constant temperature bath as well as in the heat sink. The temperature of the air was 25°C by heat exchange between the air and the circulating water. The gradient layer was painted black, and the electrical output signals were recorded on a potentiometer (SP-H5P, Riken Denshi). The difference in air temperatures between inlet and outlet of the calorimeter (ΔT) was recorded on a chart. From the rate of air flow and ΔT, one can calculate the sensible heat loss into the air. At a calorimeter temperature of 25°C, ΔT was less than 0.5°C and heat loss through this avenue was less than 1% of the total heat loss in rats. The sum of the heat loss recorded by the gradient layer and that lost into the air was expressed as the total dry heat loss (\(\dot{H}_{\text{d}}\)). The evaporative heat loss from the animal (\(\dot{H}_{\text{w}}\)) was calculated from relative humidity of the air leaving the calorimeter and the rate of air flow. Relative humidity of the air leaving the calorimeter was measured by a humidity sensing device (VAISALA HMI-11, Takara-Vaisala) installed in an outlet tubing. The air from the calorimeter was collected into a reservoir rubber bag (5 liters) for 3 min at intervals of 5 min. A small portion of air (200 ml/min) from the bag was introduced to a Beckman E2 oxygen analyzer. Heat production (\(\dot{M}\)) was calculated from the \(\dot{V}_{\text{O}_2}\) and the calorific equivalent of 4.8 kcal·liter\(^{-1}\) for oxygen consumed (DAWSON et al., 1970). In the present experiment, both \(\dot{M}\) and \(\dot{H}_{\text{d}}\) were expressed in W·m\(^{-2}\) (body surface area m\(^2\) =

![Fig. 2. Calibration curves of the calorimeter at 3 different calorimeter temperatures.](image)

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The gradient layer of the calorimeter was calibrated with an electric heater made of a constantan wire. Figure 2 shows the calibration curves for three different calorimeter temperatures (15, 25 and 35°C) with an air flow rate of 1.4 liters/min. With changing calorimeter temperature from 15 to 35°C, the slope of the curve expressed in mV/W increased but insignificantly (less than 4%). The curves were linear at all calorimeter temperatures. These data indicate that the calorimeter used in this experiment was a high quality instrument to measure heat loss at wider range of calorimeter temperature. Wires for colonic temperature recordings were led out through a small 6-pin socket on the door. A stainless wire mesh cage (8 x 8 x 18 cm in size) was placed inside the calorimeter whenever the measurement was made. Rats were forced into a rather tightly fitting wire mesh cylinder (7 cm in diameter and 15 cm in length, closed at one end in cone shape) by pushing the hips with a stainless steel spring. Rats of similar size were selected in this experiment to obtain the same extent of restraining stress. A shallow aluminum square pan filled with liquid paraffin to collect feces and urine from the animal was placed under the cage or the cylinder, so as to prevent unwarranted evaporation from the wastes.

**Measurement of heat balance during restraint.** A total of 18 male Wistar rats weighing 290-300 g were used. All animals were kept at 24±2°C ambient temperature and subjected to artificial light from 6:00 to 20:00 for at least 4 weeks before the experiment. Food and water were supplied *ad libitum*. All measurements were made between 8:30 and 16:00. Prior to the experiment, the incisors of the rat were cut and a thermistor probe for colonic temperature was inserted 7 cm into the anus. The lead wire from the probe was fixed to the base of the tail by an adhesive (Alon Alpha®, Sankyo Chemical Co., Ltd.) to secure the probe in a proper position throughout the experiment. Then, the animal was transferred into the calorimeter. \( \dot{M}, \dot{H}_{dgy}, \dot{H}_{w}, \text{and } T_{co1} \) were measured every 5 min. Approximately 2 hr after thermal equilibrium was obtained (pre-restraint period), the rat was quickly taken out of the calorimeter and was restrained with the wire mesh cylinder described above. The animal was, again, quickly returned into the calorimeter and the heat balance was measured for the following 2.5 hr (restraint period). Thereafter, the rat was free from the restraint in the calorimeter (post-restraint period).

To study the effect of sympathectomy and adrenalectomy on energy metabolism in rats during restraint, the following experiments were performed. Twelve rats, which had not been used for the heat balance measurement described above, were divided into 2 groups. One day prior to the experiment, chemical sympathectomy was produced by injection of 6-hydroxydopamine HBr (6-OHDA, 100 mg/kg i.p., Wako Pure Chemical Industries, Ltd.) in 6 rats (6-OHDA group). In another 6 rats, bilateral adrenalectomy was performed 2 days prior to the experiment (ADREX group). In these 2 groups, changes in heat production and heat.
HEAT BALANCE IN RESTRÄNTION

loss during restraint were measured with the direct calorimetric method described above. Instead of using the humidity sensing device (VAISALA HMI-11), the moist air from the calorimeter was passed through U-tubes which contained silica gel, so that the evaporative water loss was directly measured by the increased weight of the U-tubes every 15 min. The statistical significance of the results was tested by the Student t-test.

RESULTS

Table 1 and Fig. 3 show the averaged results obtained from 6 rats by direct calorimetry at a calorimeter temperature of 25°C. In the pre-restraint period, the rate of total heat loss ($H_L = H_{L, dry} + H_{L, wet}$) was nearly same as that of heat production ($M$). The rate of heat storage ($S$) was nearly zero and a thermal equilibrium was obtained at this calorimeter temperature. $T_{o,1}$ was maintained at a level of 37.4°C (37.42°C±0.12 SE–37.40°C±0.07 SE). At the beginning of restraint, $M$ increased sharply and significantly, from 53.86 W·m⁻²±3.12 SE to 69.12 W·m⁻²±2.86 SE. The high value of $M$ was maintained throughout the restraint period. $H_L$, which was 54.02 W·m⁻²±2.78 SE at the beginning of restraint, increased progressively and reached a high value of 67.44 W·m⁻²±2.48 SE at 40 min of restraint period. The increase in $H_L$ was largely due to an increase in $H_{L, dry}$. During the first 40 min of restraint, $T_{o,1}$ increased from 37.40°C±0.07 SE to 38.03°C±0.16 SE. The change in body heat content ($\Delta S$) calculated by integrating $S$ during this period of time,

$$\Delta S = \int_{t_0}^{40} S \, dt$$

was approximately 4.3 kcal·m⁻². The change in the mean body temperature of the rats ($\Delta T_b$) calculated by the following equation,

$$T_b = \frac{\Delta S}{\text{mass} \times \text{specific heat} (0.83 \text{ kcal·kg}^{-1}·\text{°C for rats (HART, 1951))}}$$

Table 1. Changes in heat balance in 6 rats at a calorimeter temperature of 25°C.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>5</th>
<th>40</th>
<th>5</th>
<th>40</th>
<th>140</th>
<th>5</th>
<th>40</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M$ (W·m⁻²)</td>
<td>52.36</td>
<td>53.86</td>
<td>69.12</td>
<td>68.55</td>
<td>71.68</td>
<td>57.53</td>
<td>54.68</td>
<td>57.18</td>
</tr>
<tr>
<td>±3.65</td>
<td>±3.12</td>
<td>±2.86</td>
<td>±2.77</td>
<td>±3.10</td>
<td>±3.22</td>
<td>±3.08</td>
<td>±2.64</td>
<td></td>
</tr>
<tr>
<td>$H_L$ (W·m⁻²)</td>
<td>53.86</td>
<td>54.59</td>
<td>54.02</td>
<td>67.44</td>
<td>68.12</td>
<td>63.27</td>
<td>60.88</td>
<td>57.12</td>
</tr>
<tr>
<td>±2.11</td>
<td>±1.24</td>
<td>±2.78</td>
<td>±2.48</td>
<td>±2.76</td>
<td>±2.24</td>
<td>±1.86</td>
<td>±1.93</td>
<td></td>
</tr>
<tr>
<td>$S$ (W·m⁻²)</td>
<td>-1.50</td>
<td>-0.73</td>
<td>15.10</td>
<td>1.11</td>
<td>3.56</td>
<td>-5.74</td>
<td>-6.20</td>
<td>0.06</td>
</tr>
<tr>
<td>±1.36</td>
<td>±1.24</td>
<td>±2.53</td>
<td>±2.22</td>
<td>±2.38</td>
<td>±2.37</td>
<td>±2.38</td>
<td>±1.33</td>
<td></td>
</tr>
<tr>
<td>$T_{o,1}$ (°C)</td>
<td>37.42</td>
<td>37.40</td>
<td>37.68</td>
<td>38.03</td>
<td>38.12</td>
<td>38.13</td>
<td>37.95</td>
<td>37.78</td>
</tr>
<tr>
<td>±0.12</td>
<td>±0.07</td>
<td>±0.18</td>
<td>±0.16</td>
<td>±0.13</td>
<td>±0.19</td>
<td>±0.13</td>
<td>±0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SE. $N$=6. Time indicates minutes in each condition.

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Fig. 3. Heat production, heat losses and colonic temperature in pre-restraint, restraint and post-restraint at a calorimeter temperature of 25°C. Thin vertical columns indicate interruptions of measurement by transferring rats from one condition to the other. Vertical broken line indicates the time after which thermal equilibrium during restraint is maintained. Small vertical lines are standard errors. Number of rats were 6, each rat being used only once.

was 0.7°C, which was close to the change in body temperature actually measured during this period. After 40 min of restraint, \( H_t \) tended toward a steady high value, and \( S \), which was positive during the first 40 min, became nearly zero. These results indicated that a thermal equilibrium was reached during a prolonged restraint at a calorimeter temperature of 25°C. \( T_{col} \) increased only by 0.09°C during the last 110 min of restraint. When the rats were freed from restraint, \( \dot{M} \) and \( \dot{H}_L \) decreased progressively. However, for the first 10-20 min of post-restraint period, the rats moved around and eagerly groomed themselves in the calorimeter. The decrease in \( \dot{M} \) during post-restraint period was not prominent in this very beginning of post-restraint period. After 20 min of post-restraint period, \( \dot{M} \) decreased sharply and \( S \) became negative. Sixty minutes after release from the restraint, \( S \) became nearly zero and \( T_{col} \) reached a steady value of 37.78°C \( \pm 0.09 \) SE.

Figure 4 shows the changes in \( \dot{M} \) and \( \dot{H}_L \) by 2.5-hr restraint in normal, 6-OHDA and ADREX groups. In normal control group, as shown in Fig. 3 and Table 1, the mean \( \dot{M} \) and \( \dot{H}_L \) were significantly higher during restraint than in either the pre- or post-restraint period \( (p<0.01) \). In 6-OHDA and ADREX groups, the pre- and post-restraint values in \( \dot{M} \) and \( \dot{H}_L \) were significantly smaller.
Fig. 4. Mean $\bar{H}_L$ and $\bar{M}$ (with standard errors) in pre-restraint (left open columns), restraint (shaded columns) and post-restraint (right open columns) rats at a calorimeter temperature of 25°C. CONTROL, 6 normal rats; 6-OHDA, 6 6-OHDA-treated rats; ADREX, 6 adrenalectomized rats. ** $p<0.01$.

than those in normal control group ($p<0.01$). With restraint, either a very small (6-OHDA group) or no increase (ADREX group) in $\bar{M}$ and $\bar{H}_L$ was observed.

DISCUSSION

At a calorimeter temperature of 25°C, restraint caused an immediate increase in heat production ($\bar{M}$) in rats. Heat loss ($\bar{H}_L$) increased progressively and reached a high value, which was approximately same as that of $\bar{M}$, at 40 min of restraint. During the initial 40 min of restraint, the rate of heat storage ($\dot{S}$) was positive and the change in body heat content ($\Delta S$) became approximately 4.3 kcal·m$^{-2}$. During this period of restraint, colonic temperature ($T_{col}$) increased from 37.4 to 38.0°C, which was close to the mean body temperature calculated by $\Delta S$, body mass and specific heat for rats. $\bar{M}$ and $\bar{H}_L$ were maintained at nearly same high values and $\dot{S}$ became zero during the rest of restraint period. $T_{col}$ did not change significantly in this period of restraint (38.03–38.17°C). Restraint-hypothermia, repeatedly reported in slightly cold environments as has been summarized in the monograph by Hart (1972), might have been elicited by tying the animals in a "spread-eagle" fashion, which should have greatly increased nonevaporative heat loss from an enlarged effective body surface area.

The rats, struggled only at the very beginning of restraint, were relatively quiet in the rest of restraint period. Therefore, it seemed unlikely that the increased muscular activity was the only cause of this increased heat production during restraint. McEwen, Jr. (1975) observed thermoregulatory responses of restrained rabbits at subneutral ambient temperature. Ear skin temperature was not significantly different between the restrained and unrestrained rabbits, whereas heat production and heart rate were significantly higher in the restrained animals. As to the cause of this increased metabolism during restraint, he suggested an
emotional factor such as fear by which secretion of catecholamines might be increased to a great extent. By using a chronic indwelling catheter to draw a small amount of blood without any physical or emotional stress, BÜHLER et al. (1978) confirmed a very high plasma concentrations of catecholamines in rats and rabbits during a short period of restraint. According to them, the restraint increased significantly all three catecholamine levels with approximate increases of 2,000% for adrenaline, 600% for noradrenaline and 300% for dopamine compared to the basal values in the free-moving rats. Since catecholamines are the hormones for eliciting strong calorigenic response in mammals, an increased plasma concentration of these hormones may be concerned with the marked rise in heat production during restraint. POHOSKA et al. (1975) observed an increase in urinary adrenaline excretion and suggested a participation of adrenaline in the restraint-hypermetabolism in rats. As shown in Fig. 4, the increased heat production in restraint was greatly inhibited after the chemical sympathectomy with 6-hydroxy-dopamine. The restraint-hypermetabolism was completely abolished by the bilateral adrenalectomy in rats. These results suggest the importance of catecholamines in maintaining a high metabolism in these animals.

In spite of the increased $T_{co1}$ by 0.7°C after restraint, an evaporative thermolytic change, such as saliva spreading, was not observed. The averaged change in total heat loss, which was largely attributed to $H_{L, dry}$, was same as that of heat production; in other words, thermal equilibrium was maintained in these animals even with a higher core temperature than in normal unrestrained animals. These changes resemble those observed during muscular exercise in man (CHAPPUIS et al., 1976), and give an impression that the set point of body temperature may shift during physical restraint in rats. Since it is generally accepted that the elevated body temperature during exercise is rather independent of environmental temperature (NIELSEN, 1938; CHAPPUIS et al., 1976), a further observations should be made on restrained rats under various environmental conditions. Many studies on temperature regulation have used restrained animals assuming that restraint has little effect on temperature regulatory responses. However, as shown in this study, restraint may induce different thermoregulatory responses due to an elevation of core temperature in the animals. Whenever the study on thermoregulatory response is performed in the restrained animals, one should always take those into grave consideration.

This report has also described a direct calorimeter for small animals, built in our laboratory. As other gradient layer calorimeters (BENZINGER and KITZINGER, 1963; HAMMEL and HARDY, 1963; SPINNER et al., 1973; POPPENDIEK et al., 1976), the calorimeter has a high sensitivity and a short response time by using thin gradient layers and maintaining a constant temperature of the heat sink at a very constant level of ±0.01°C. Since a large amount of temperature controlled water is circulated through the calorimeter heat sink, and temperature of the air sent into the calorimeter is precisely controlled at a set temperature by heat exchange.
between the air and the circulating water, the effect of changes in room temperature can be avoided as shown in Fig. 2. In other words, the system can be used in the laboratory of which air temperature is controlled by ordinary air conditioning facilities. The actual operation of the system is relatively simple. The calorimeter reported here is a useful instrument to measure heat balance in small animals over a wide range of temperature, and does not interfere with the animal’s vasomotor, respiratory, behavioral and diurnal activities.

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REFERENCES


