Temperature sensitivity of skeletal muscle in the conscious goat

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Jessen, Claus, Gero Feistkorn, and Alfred Nagel. Temperature sensitivity of skeletal muscle in the conscious goat. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54(4): 880-886, 1983.—A method has been developed to test the hypothesis that the deep tissues of the legs, e.g., skeletal muscle and/or periosteum, contain thermosensitive elements feeding signals into the temperature-regulating system. Stainless steel thermodes of 10 to 12-mm diameter and 100 to 150-mm length were chronically implanted into the marrow spaces of both humeri and femora. The temperature of the deep muscle layers was altered by perfusing the thermodes with water of 0°C. This caused respiratory evaporative heat loss to decrease by 0.15-0.20 W/kg. The small but significant response occurred at constant general body temperature and is therefore indicative of a local effect of the cooling on deep thermosensitive elements in the legs themselves and a neural afferent transmission of temperature signals into the temperature-regulating system.

muscle thermoreceptors; temperature regulation; control of heat loss

It has been frequently postulated that muscle thermoreceptors participate in control of body temperature (15, 17). So far, only circumstantial evidence has been available in favor of or against the existence and action of muscle thermoreceptors. This study describes experiments performed to isolate a fraction of the total musculature thermally and to correlate thermal responses of a conscious animal with experimentally induced alterations of temperature in these muscles. The experimental approach rests on a distinct feature of the goat's skeleton. In this species, the large bones of the upper limbs, the humerus and femur, have wide cavities and thin walls. Thermodes implanted into the marrow spaces of both humeri and both femora were employed to alter the temperature of the muscles surrounding the bones, which could be a sufficiently large fraction of the total musculature to test the question of thermal afferents. To restrict the thermal stimuli to the legs the animals were further equipped with intravascular heat exchangers (5), serving to keep the temperature of the body, excluding the legs, constant during periods of altering inner leg temperature.

Methods

Animals and Preparations

The experiments were performed on four female Toggenburg goats whose approximate body weights and ages were 35 kg and 2 yr, respectively. The "Guiding Principles in the Care and Use of Animals" were observed. Prior to the experiments, the following instruments were implanted chronically into the animals.

Bone thermodes. In four separate sterile operations extending over a period of 2 or more mo, stainless steel thermodes of 100-150-mm length and 10-12-mm diameter (Fig. 1) were implanted into the proximal bones of all four limbs (humeri and femora). The marrow space was opened at the tuberculum majus and the fossa intertrochanterica (9), respectively, and the content of the cavity was removed. The thermode was then fully embedded in the marrow space. After recovery from the operations, the thermodes did not interfere with normal locomotion and even permitted experiments on a treadmill (not reported in this study). During the experiments, the thermodes were connected, by means of flexible percutaneous tubes, to an external perfusion circuit. By applying a pressure gradient of 4 bar between inlet and outlet a flow rate of approximately 4 l/h per thermode was obtained.

Intravascular heat exchanger. This consisted of three pairs of U-shaped polyethylene tubes, which were inserted into the venous vascular system and extended from the jugular vein to the iliac bifurcation. Design and implantation of the tubes have been previously described (5). By controlling the perfusion inlet temperature and perfusion flow rate, body temperature could be raised, lowered, or, within limits of ±0.1°C, held constant at any required value.

Para-aortic thermocouple guide tube. The tip of a blind-ending polyethylene tube was sutured to that side of the abdominal aorta which was reflected from the vena cava and served as a guide for a thermocouple. The temperature measured at the tip of the tube (Tทะ) was considered as an indicator of the temperature of the arterial blood and to reflect body core temperature (2). At least 2 wk elapsed between the last operation and the first experiment.

Experiments

Apart from a few pilot experiments (Figs. 2, 3, and 7), 80 experiments were performed in a climatic chamber at an air temperature of 38°C and a relative humidity of 0161-7567/83/0000-000081.50 Copyright © 1983 the American Physiological Society

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30% (dew point, 18°C) according to the following protocol. Forty-eight hours before the beginning of an experiment the animal was deprived of food. Fourteen hours before, the animal was brought into the climatic chamber. At 8 A.M. the perfusing system and measuring probes were attached to the animal. Following a free interval of at least 1 h the first of four experiments, which were performed in 1 day, commenced. Each experiment lasted 90 min, during which time general body temperature was kept constant, by means of the intravascular heat exchanger, at a predetermined level between 39 and 42°C. From the 30th to the 60th min the leg thermodes were perfused with a liquid of 0°C. Two successive experiments were separated by a free interval of 10- to 30-min duration.

Recordings

General body temperature was measured at the wall of the abdominal aorta (T\textsubscript{a}). In one experiment (Fig. 2) deep leg temperatures were measured at various distances from the femur. The rate of heat transfer via the bone thermodes was calculated from measurements of inlet and outlet temperatures and the perfusion flow rate. All temperatures were measured by thermocouples and continuously recorded on a multipoint recorder. The accuracy of measurement was within ±0.1°C.

An open-circuit respiratory system was used to calculate heat production (M) and respiratory evaporative heat loss (REHL). The airflow rate was measured by a dry gas meter (±1%) and corrected to STPD. The difference in \textit{O}_2 content between inlet and outlet air was calculated from continuous recordings of the \textit{O}_2 partial pressure (Servomex OA 184). A constant respiratory quotient of 0.85 was assumed. \textit{O}_2 consumption was converted to \textit{M} by using 20.549 kJ/l as a caloric equivalent for \textit{O}_2. The difference in water content of inlet and outlet air was measured by continuous recordings from an infrared analyzer (URAS, manufactured by Hartmann & Braun, FRG). The increase in water content was converted to REHL by using 2.411 kJ/g as the latent heat of vaporization of water.

RESULTS

Effects of Leg Cooling on Inner Leg Temperature and Thermal Balance

One experiment was performed to assess the temperature field within and around the bone. Five thermocouples were placed perpendicularly to the longitudinal axis of the femur (Fig. 2). One thermocouple was located between the inner surface of the bone and the outside of the thermode. A second thermocouple lay at the outer surface of the bone, and three further couples were within the muscle at distances between 4 and 14 mm from the outer surface of the bone. The left side of Fig. 2 shows the time course of the temperatures at these locations during a perfusion period performed with the standard cooling intensity of 0°C. Within 40 min the temperature in the cavity of the bone fell to 16°C. At the peristome, the temperature was 26°C. However, the thickness of the bone at the location of the thermocouple was larger than at other radii. As a consequence, the change of muscle
temperature at this lateral side was smaller than at corresponding sites of the medial side, where muscle temperatures decreased to 21 and 31°C, respectively. The temperature changes at these sites have to be seen in relation to the oval cross section of the proximal limb, the diameters of which varied between 100 and 150 mm. Therefore, the induced alterations of muscle temperature were limited to the deep layers of muscle close to the bone, and no temperature changes at subcutaneous layers could be observed.

Figure 3 shows the effect on the animal’s thermal balance of cooling all four proximal limbs. From the 24th to the 56th min the thermodes were perfused with a liquid of −10°C inlet temperature. The resulting heat loss amounted to 2.65 W·kg body wt−1 and caused general body temperature to drop initially by 0.1°C. This was followed by vigorous shivering, which increased $M$ from 2.8 to more than 5 W·kg−1. Thus heat loss via the thermodes and excess heat production were close to balance, and general body temperature ($T_{\text{pa}}$) did not consistently deviate from its control level during the period of leg cooling. After the leg cooling had been finished, $M$ slowly returned to its control level.

In this experiment the heat transfer from the body via the thermodes amounted to approximately 1.5 times the $M$ of a resting goat in a thermoneutral environment. These data complement the results of the temperature measurements in the muscle (Fig. 2) and demonstrate that bone thermodes present an efficient means of heat exchange with the deep tissues of the legs.

**Effects of Isolated Leg Cooling on REHL**

In a series of pilot experiments, leg cooling often decreased $T_{\text{pa}}$ below its control levels. Therefore, in all further experiments intravascular heat exchangers were employed to prevent $T_{\text{pa}}$ from falling and to restrict the effect of the bone thermodes to the legs themselves.

Figure 4 shows the time course of a single experiment at an air temperature of 38°C and a relative humidity of 30%. Throughout the full length of the experiment $T_{\text{pa}}$ was maintained, by means of the intravascular heat exchanger, between 41.7 and 41.9°C. Due to the combined internal and external heat load, the animal panted vigorously, and REHL during the initial control period was between 2.2 and 2.3 W·kg−1. From the 30th to the 60th min, all four legs were cooled with a perfusion temperature of 0°C. This caused REHL to decrease to 1.9 W·kg−1. In the third part of the experiment, the legs were warmed up to $T_{\text{pa}}$ and REHL returned to its control level. The experiment shows that cooling the deep tissues of the legs can withdraw part of the temperature signals driving REHL.

Following an identical protocol, 80 experiments were performed in four animals. The results of all 20 experiments in one animal are shown in Fig. 5. $T_{\text{pa}}$ was maintained at preset levels between 40.0 and 41.7°C. The mean value of the last 10 min of all control periods was 40.8 ± 0.1°C (SE). Separately for each period, the temperatures at all instants in 2-min intervals were calculated as differences between the actual temperature and
which $T_p$ during leg cooling equaled or exceeded the temperature during the initial control period. The results of these 30 experiments are shown in Fig. 6. The general pattern of the response is similar to that shown in Fig. 5.

FIG. 3. Time course of experiment in conscious goat showing effect of leg cooling on heat production ($M$) and general body temperature ($T_p$). Other abbreviations as in Fig. 2.

Due to the control by the intravascular heat exchanger, $T_p$ remained stable throughout the periods. The mean deviation from the reference level never exceeded 0.06°C, and during leg cooling $T_p$ was on the average 0.01°C higher than during the last 10 min of the control period. However, during leg cooling REHL decreased by approximately 0.15 W·kg$^{-1}$, indicating that the cooling induced this effect directly and locally in the legs themselves. After the cooling periods had been finished, REHL gradually increased and finally exceeded its control level.

The Wilcoxon matched-pairs signed-rank test was used to compare $T_p$ and REHL during the periods of leg cooling with the values of the initial and final control periods. While $T_p$ values were not significantly different, REHL was significantly lower during leg cooling than during the initial and final control periods ($P < 0.01$, two-sided test). When the results of all 80 cooling periods in the four animals were treated in the same way, $T_p$ during the cooling periods turned out to be significantly lower than during the control periods ($P < 0.05$). Although the mean difference did not exceed 0.04°C, it was decided to restrict further consideration to those experiments in which $T_p$ during leg cooling equaled or exceeded the temperature during the initial control period.

FIG. 4. Time course of experiment in conscious goat showing effect of leg cooling on respiratory evaporative heat loss (REHL). General body temperature ($T_p$) was clamped by means of intravascular heat exchangers.

FIG. 5. Changes of respiratory evaporative heat loss ($\Delta REHL$) and general body temperature ($\Delta T_p$), when legs were subject to central cooling (30th-60th min). Body temperature was clamped by means of intravascular heat exchangers. Mean values ± SE of changes relative to average of last 10 min of control periods (solid bars). Twenty experiments in animal G 271.
During leg cooling, REHL decreased without concomitant fall in $T_{pa}$. REHL during cooling was significantly smaller than during the preceding ($P < 0.02$) and following ($P < 0.05$) control periods. $T_{pa}$ during leg cooling was not different from the first control period but significantly higher than during the second control period ($P < 0.01$).

**Effects of Isolated Leg Cooling on $M$**

In a few experiments the legs were cooled while the animal was exposed to a low thermoneutral environment and subject to mild internal cooling via the intravascular heat exchanger, which caused the animal to shiver continuously. Additional leg cooling (Fig. 7) further increased $M$ from 2.6 to 3.3 W·kg$^{-1}$. This occurred with rising $T_{pa}$ and must therefore be attributed to a local effect of the cooling in the legs themselves.

**DISCUSSION**

Recent experiments in goats have shown that a considerable fraction of the thermal afferent information signaling body temperature arises from thermosensitive sites other than the hypothalamus and spinal cord (7). As to the origin of these afferents, one conceivable site is skeletal muscle. The question of muscular thermal afferents has been frequently discussed in the past, mostly in connection with experiments in humans. Considerations have been prompted by inconsistencies in relating thermoregulatory responses to body temperatures when the

**FIG. 6.** Changes of respiratory evaporative heat loss (AREHL) and general body temperature ($\Delta T_{pa}$), when legs were subject to central cooling (30th-60th min). Body temperature was clamped by means of intravascular heat exchangers. Mean values ± SE of changes relative to average of last 10 min of control periods (solid bars). Thirty experiments in four animals.

**FIG. 7.** Time course of experiment in conscious goat showing effect of leg cooling on heat production ($M$). General body temperature ($T_{pa}$) was clamped by means of intravascular heat exchangers.
central drive was represented by local temperature at one or several sites (1, 8, 12, 13). Especially during exercise, the rate of sweating was often observed to vary before any noticeable changes of internal temperature, i.e., rectal, esophageal, or tympanic temperature, had occurred (1), and at identical combinations of peripheral and central temperatures widely different levels of sweat rate were found (12). However, the rate of sweating precisely matched the rate of work (12), which implies that the latter was somehow reflected in the input to the controller. This could be a nonthermal input, originating from mechanoreceptors in muscles and joints (1, 12), or a thermal input, originating from thermoreceptors in or near the exercising muscles (10). The temperatures of active muscles and of their venous drainage better than any other temperatures paralleled the rate of skin sweating (10), although others found it impossible to assign a unique role to either rectal or muscle temperatures in the control of regulatory sweating (11). Thus muscle thermoreceptors have become and long remained a postulate of thermoregulatory models (15, 17). The lack of direct experimental evidence in favor of or against muscular thermoreceptors was primarily due to the fact that no method was hitherto available to thermally isolate a sizable fraction of the musculature. If thermoreceptors reside in all skeletal muscles, the thermal input provided by any small part of the musculature must be assumed to be a correspondingly small fraction of the total thermal input. Therefore, generation of a thermal response large enough to be measured in a conscious animal requires thermal isolation and selective stimulation of a relatively large part of the musculature. The bone thermodes in combination with the intravascular heat exchangers used in this study appear to have fulfilled this requirement and created a situation which, on one hand, is totally unnatural and without any resemblance to normal temperature distribution within the body, but which, on the other hand, provides a direct experimental approach to the question of muscular thermoreceptors. During the periods of leg cooling respiratory evaporative heat loss showed a small but statistically significant reduction. Since side effects of leg cooling on general body temperature were offset and the temperature of all known thermosensitive sites of the body either remained stable or even increased during the period of leg cooling, the decrease of respiratory evaporative heat loss is indicative of a local effect of the cooling on deep thermosensitive elements in the legs themselves and a neural afferent transmission of temperature signals. The decrease of heat loss can then be interpreted as a response to a partial withdrawal of warm signals driving heat-dissipating mechanisms.

It might be argued that leg cooling could have caused reflex vasoconstriction in the skin and a decrease of skin temperature, resulting in a larger input from cutaneous cold receptors. However, no temperature changes could be observed at a subcutaneous level, and most experiments were performed at an air temperature of 38°C, rendering only a very narrow margin for skin cooling to occur.

Corresponding effects of leg cooling could be observed with regard to shivering and heat production. The reversed arrangement of stimuli, i.e., leg warming at low general body temperature, proved impossible to test. Transfer of sufficient amounts of heat into the legs required perfusion temperature exceeding the pain threshold. This raises the following question: to what extent can the effect of leg cooling be mediated by pain receptors? However, no response except the thermoregulatory one was observed during leg cooling. Polymodal nociceptors in the monkey's skin do not respond to cooling (3).

Concerning the nature of the neural afferents involved in mediating the response to leg cooling, a number of pertinent studies have been published. Afferent fibers of groups III and IV represent the major part of afferents in muscle nerves; e.g., in the nerve supplying the posterior tibial muscle of the cat about 60% of all afferents were found to be nonmyelinated (14). In the anesthetized cat, about 50% of all afferents of groups III and IV of the gastrocnemius muscle responded to alteration of muscle temperature, most of them by increasing frequency with increasing temperature (4). Recordings from afferent fibers of skeletal muscle of the anesthetized rat showed thermosensitive units, the sensitivity of which could be enhanced by local administration of potassium and lactic acid (16). Thus there appears to be a neuronal counterpart to account for afferent transmission of temperature signals from the deep structures of the legs. In this context it may be noted that neither our own experiments nor the neuropathological studies mentioned above could discriminate between afferents from the muscle and the periosteum. The periosteum is known to contain pain receptors, the afferent fibers from which are considered part of the nerves supplying the muscles.

The thermoregulatory responses to leg cooling were small. The effect of cooling all four proximal limbs with a perfusion temperature of 0°C was approximately equivalent to that of a 0.15°C decrease in general body temperature. This may either indicate a still relatively small fraction of the musculature affected by the bone thermodes or reflect a low density of thermosensitive elements in the deep tissues of the legs. However small the responses were, the results of this study appear to have provided evidence in favor of thermal afferents from the deep structures of the legs and to support the concept of a general distribution of thermosensitive elements throughout the body (8).

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REFERENCES

3. CROZE, S., R. Duclaux, AND D. R. KENSHALO. The thermal


