Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming

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The preoptic/anterior hypothalamus (POAH) plays a central role in temperature regulation; preoptic/anterior hypothalamic temperature; heat production

Ful1er, C. A., B. A. Horwitz, and J. M. Horowitz. Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. Am. J. Physiol. 228(5): 1519-1524. 1975.—The concurrent neural control of two thermoregulatory responses, shivering thermogenesis (ST) and nonshivering thermogenesis (NST), was investigated in chronically implanted cold-exposed rats. The effects of heating the preoptic/anterior hypothalamus (POAH) on shivering and on the rate of oxygen consumption (V\text{O}_2) were measured in these unanesthetized animals. With ambient temperature maintained constant (at some value between 10 and 16°C), warming the hypothalamus 2-3°C resulted in a significant decrease in V\text{O}_2 (P < 0.001) and an increase in shivering (P < 0.01), these responses being reversed on cessation of hypothalamic warming. These results are consistent with the proposal that, in the cold-exposed animal, elevated POAH temperatures directly inhibit NST even though shivering may increase (possibly as a compensation for the decrease in nonshivering heat production). They also rule out the possibility that, in the rat, signals from cutaneous and hypothalamic thermoreceptors are integrated in an identical manner by the neural controllers for ST and NST.

temperature regulation; preoptic/anterior hypothalamic temperature; heat production

The present study was thus undertaken in an attempt to determine which of the models is applicable to cold-exposed rats: i.e., whether or not changes in hypothalamic temperature are associated with parallel control of both shivering and nonshivering heat production.

METHODS

Chronic implantation. Ten male, Long-Evans hooded rats (350-450 g) were anesthetized lightly with ether, weighed immediately, and injected intraperitoneally with sodium pentobarbital, 45 mg/kg. Atropine sulfate was administered at a dose of 0.02 mg/kg ip. The level of anesthesia was maintained by subsequent doses of 3 mg sodium pentobarbital injected intraperitoneally. During surgery, the rat was positioned on a heating pad, and its colonic temperature was maintained between 37 and 38°C.

The upper back and skull of the animal were shaved, and an initial incision made between the scapulae was extended anteriorly for a distance of approximately 3 cm. A pair of electromyographic (EMG) electrodes with a fixed distance of 5 mm between the two wires was inserted in the platsyma and/or acromiotrapezius muscle, and the electrodes were sutured into place. (This site was selected as showing shivering activity representative of that seen throughout the musculature (18, 26).) A loop of lead wire from the electrodes was inserted into a skin pouch to provide slack for

1 By definition nonshivering heat production refers to heat produced from pathways that do not involve muscular contraction (18).
movement of the animal. The ends of the wire were exteriorized through a skin incision over the skull.

The rat was then positioned in a stereotaxic frame and an incision was made along the midline plane of the skull in an anterior-posterior direction. The skin was retracted laterally and the bone exposed along the length of the sagittal suture. Four holes were drilled through the skull: three for set screws to anchor an electrical connector and the fourth for insertion of a thermistor through the skull. Two of the set screws were posterior and lateral to bregma; the third was anterior and lateral to bregma. The third set screw was also utilized as a reference electrode. The thermistor (Vero 32A12, 2,000 Ω) was placed 5 mm below a point on the surface of the brain located on the coronal suture and approximately 1 mm lateral to bregma. Dental cement was used to secure the thermistor to the skull. The final placement of the stereotactically implanted thermistor was: A 5.6 mm, L 1.0 mm, and V − 2.7 mm as described by the König and Klippel atlas (20).

An ITT Cannon electrical connector (MD1-9SL1) was soldered to the EMG electrode leads, the thermistor, and the ground wire. The plug was permanently mounted to the top of the skull with dental cement. Incisions were closed, the animal was removed from the stereotaxic frame, and the sutured incisions were sprayed with an aerosol topical antibacterial agent, Topazone. The rat was allowed to recover at 23 ± 1°C for a period of at least 4 days (at which time he had begun to regain body weight).

Measurements of temperature, shivering, and oxygen consumption. Hypothalamic temperature was measured by placing the leads from the implanted thermistor in one arm of a Wheatstone bridge. As the temperature of the thermistor changed its resistance, the bridge became unbalanced and the voltage across two nodes of the circuit was altered. This voltage was recorded on a Houston 3000 penwriter.

Warming of the hypothalamus was accomplished by applying a voltage across the implanted ceramic thermistor. This voltage was sufficient to raise the temperature of the thermistor 2-3°C as determined in testing prior to implantation. However, within the brain the temperature of the tissue would be warmest near the thermistor (6).

To quantitate shivering, a function generator (Hewlett Packard 3300A), triggered by the amplified shivering bursts, was utilized to produce a square-wave pulse of sufficient duration to be recorded as a clear deflection by the penwriter. This pulse, which was generated by amplified bursts of shivering exceeding a threshold of 0.1 V, had a duration of 200 ms. Thus, each shivering burst (lasting on the average approximately 400 ms) was counted as a single event (see RESULTS). A monitor oscilloscope (Tektronix RM 564) served to verify that the pulses did indeed correspond to shivering bursts.

Heat production was measured indirectly by monitoring oxygen consumption. The unanesthetized rat was lightly restrained by placing it in an acrylonitrile holder (Econocage). This holder was then positioned in a water-jacketed chamber open only to a supply of 100% oxygen contained in a Med-Science Electronics volume meter (model 160). Ambient temperature was controlled via the surrounding water jacket and expired CO2 was absorbed from the chamber with Baralyme (barium hydroxide lime). The sides and bottom of the water jacket were lined with Styrofoam for thermal insulation and this Styrofoam was in turn covered with aluminum foil for electrical isolation.

Data acquisition. Prior to the day of the experiment, the animal was shaved if this had not already been done within the previous 10-day period. Immediately before the experimental trial, the rat was anesthetized lightly with ether and the cemented skull plug was joined to a previously wired Cannon electrical connector (MD1-9PL1). A Yellow Springs Instrument Co. (YSI) thermistor was inserted 6-8 cm into the colon and the rat was then placed in the acrylonitrile restrainer. One YSI telethermometer, model 43TA, was used to record colonic temperature and a second (model 43TD) was connected to a YSI thermistor placed within the chamber housing the rat. Hypothalamic and colonic temperatures were monitored as the animal recovered from the ether. The experiment was not begun until the temperatures of both regions had stabilized at a constant level for at least 10-20 min.

For each experiment, oxygen consumption was initially measured at a room temperature between 24 and 28°C to establish a stable base line of metabolic activity. After this, the temperature of the water jacket was rapidly lowered and the rat chamber was held at a constant temperature between 10 and 16°C. Approximately 40 min after the onset of cold exposure (during the final 10-15 min of which the monitoring of shivering activity was initiated), the temperature of the hypothalamus was raised 2 3°C. Heating continued for 10-20 min and was followed by a 10- to 20-min period of nonheating. If the colonic temperature of the rat were stable, a second trial was initiated (i.e., 10-20 min of hypothalamic heating followed by a comparable period of nonheating) at the end of which the experiment was terminated. When the colonic temperature of the rat did not stabilize during the nonheating period of the first trial, the experiment was terminated without exposing the rat to hypothalamic rewarming.

Placement of thermistor. The anatomical location of the thermistor was determined in some rats by skull X rays (Fig. 1); in others, placement was ascertained with standard histological procedures. For the latter, an anesthetic dose of sodium pentobarbital was administered and 10% Formalin was perfused through the aorta. The brain was removed and stored in buffered Formalin; after a minimum 24-h period, the tissue was dehydrated and embedded in paraffin. Serial coronal sections, 15 μm thick, were stained, mounted on slides, and examined microscopically.

RESULTS

The EMG potentials generated from shivering bursts differed considerably from base-line nonshivering activity (Fig. 2, A, B). Tracings of both EMG potentials and the output of the function generator were displayed on the oscilloscope (Fig. 2, C, D) to verify that a burst of shivering elicited an associated pulse. Examination of the shivering bursts indicated that they were graded in amplitude and only those bursts that exceeded a threshold amplitude were counted. (The amplitude and duration of each shivering burst, other variables (12), were not quantitated.)

As illustrated by the example shown in Fig. 3, exposure of the rat to cold was followed by an increase in oxygen consumption and shivering activity. Upon hypothalamic
warming, the oxygen consumption of the rat apparently decreased while shivering increased. Since shivering is only a small component of the total oxygen consumption record, statistical correlations were necessary to evaluate the interaction of the shivering and $\dot{V}_O_2$ records during and after hypothalamic warming. Considering all trials (Fig. 4), the $\dot{V}_O_2$, which averaged $27.6 \pm 0.8$ ml/min·kg$^{0.73}$ ($n = 23$) during the 5-min control period prior to hypothalamic warming, was seen to decrease $5.6 \pm 1.4\%$ (relative to control values) during the first 5 min of hypothalamic warming. The differences observed between these two experimental conditions were significant ($P < 0.001$) as determined by the Student $t$ distribution with paired observations. The depressant effect during warming was reversed on cessation of heating, with the mean $\dot{V}_O_2$ after hypothalamic warming being equal to $28.6 \pm 0.7$ ml/min·kg$^{0.73}$ ($n = 20$). Moreover, this warming-induced decrease in oxygen consumption was repeatable for the same rat on reheating of the hypothalamus either immediately after the first trial (as illustrated in Fig. 3) or on a subsequent day.

Shivering was recorded during 12 experimental trials (performed on 6 rats; Fig. 4) and in all trials was always observed to be present during cold exposure. The shivering bursts consisted of very high-frequency potentials (250–300 Hz) and were approximately 400 ms in duration. These recorded potentials may not represent the response of a single motor unit (23), but rather a summation of total motor unit activity. The average number of shivering bursts during the period of hypothalamic heating ($116.9 \pm 19.3$ bursts/5 min) reflects a significant increase, $P < 0.01$ ($53.6 \pm 23.3\%$ of the control activity), as determined by the Student $t$ distribution for paired observations. This response tended to reverse after cessation of heating ($3 \pm 109.3 \pm 18.4$ bursts/5 min) but could be elicited again in the same animal on hypothalamic rewarming.

**DISCUSSION**

The observation that warming the POAH of the cold-exposed rat significantly decreased the heat production (oxygen consumption) is consistent with prior findings in the dog (6, 10) and baboon (24). In previous work, however, the individual contributions of shivering and non-shivering to the total response were not quantitatively assessed; also, although several comments regarding the occurrence of shivering are reported, most appear to be based primarily on visual observation.

In contrast, in the present study shivering was measured...
Fig. 3. Effect of cold exposure and hypothalamic warming as a function of time. Included are measurements of ambient temperature ($T_A$), colonic temperature ($T_R$), rate of oxygen consumption ($V_O_2$), and shivering activity ($S$). Point $A$ represents time at which rat was cold exposed.

by counting bursts exceeding a threshold amplitude and, although hypothalamic warming decreased the total heat production of the rat, such warming did not abolish—or even decrease—the concomitant shivering activity. In fact, shivering activity during warming of the hypothalamus was significantly increased. (The reversibility of the response and repeatability on successive trials indicate that hypothalamic heating did not functionally damage the area.) The reduction of total heat production in the presence of enhanced shivering activity can best be explained as reflecting a decrease in the magnitude of nonshivering thermogenesis.

These data thus support the suggestion by Bruck and Wünnenberg (4) that nonshivering—but not shivering—

heat production is directly affected by increasing hypothalamic temperature; in terms of the two computer simulations, the data are consistent with model B2. That is, in model B2, a simplified version of which is diagrammed in Fig. 5, the neural controller requires inputs of hypothalamic and subcutaneous temperatures for the nonshivering effector output (NST), while temperature information from spinal cord and subcutaneous areas determines the shivering output (ST). Accordingly, if the magnitude of nonshivering thermogenesis were to be reduced, shivering activity could be increased via signals over the pathway shown as a dotted line in Fig. 5. Thus, if there were a decrease in heat generated by the cervical and/or interscapular brown fat pads, a resulting decrease in temperature of various critical areas (in this case, the cervicothoracic spinal cord) would stimulate shivering. This combination of a decrease in NST accompanied by increased shivering activity was indeed observed in the present experiments (Figs. 3 and 4). (Note that the NST response (at least in brown fat) appears to be rapid enough (17) to account for the fluctuations in the $V_O_2$ record.) A further test of model

Fig. 4. Effects of heating POAH on shivering and rate of oxygen consumption ($V_O_2$). Plotted are mean percent changes from levels of activity occurring in 5-min interval prior to heating. Bars represent means $\pm$ SE, with total number of trials indicated adjacent to each bar graph. Values are for time intervals of 5 min: i.e., first 5-min period during heating (heating) and 5 min interval immediately after cessation of heating (postheating).
FIG. 5. Simplified diagram of dynamic representation of model for cold-exposed animals modified from that of Brück and Wünnenberg (4). HY, SU, and C denote hypothalamic, subcutaneous, and cervical areas, respectively. Symbol X refers to output of respective temperature receptors; R to reference neurons; I to output after comparison of X and R values. After such comparison, I values are paired as indicated and channeled to their respective signal multipliers. Within signal multipliers, MIN1 and MIN2 are threshold values for the two effector signals, YNST (for nonshivering thermogenesis) and YST (for shivering thermogenesis). Two major effectors for heat production are shown as muscle for both shivering and nonshivering heat production and brown adipose tissue (BAT) for NST. Dotted pathway illustrates proposed influence on spinal thermosensors of NST from brown adipose tissue. In this case, heat transfer from brown fat into cervical spinal region is mediated by venous drainage of fat pads and is basis for this feedback pathway (dotted line). See text for additional details.

B2, namely examination of the responses to spinal cord warming, is currently underway.

The interpretation that signals from thermoreceptors in various locations of the rat are converging on different thermoeffector systems is consistent with the work of Nutik (22) and that of Brück and Wünnenberg (4). In the latter study (4), hypothalamic warming of young (1-wk-old), cold-adapted guinea pigs at an ambient temperature of 25°C was followed by a decrease in oxygen consumption with no observed effects on shivering. However, unlike the situation in the present experiments, it appears that the guinea pigs may not have been cold stressed, and therefore, no base-line levels of shivering were available for evaluating the effects of hypothalamic heating. Thus, the results from the cold-exposed rats indicate that warming of receptors that channel signals to a specific area for control of NST may indirectly affect ST (e.g., via the feedback pathway shown as a dotted line in Fig. 5). The extent to which these data can be generalized to include other mammals is not clear since the necessary information (i.e., the effect of receptor warming on both ST and NST in cold-exposed animals) is not yet available. For example, although warming the hypothalamus of a dog maintained at an ambient temperature of 22 ± 0.5°C appeared to suppress shivering (14) no measure of NST was obtained.

In support of an alternate theory—that the increased hypothalamic temperature may control shivering directly—is the finding that in anesthetized cats electrical stimulation of various hypothalamic and midbrain areas leads to changes in shivering activity (2, 13, 27). Nonetheless, electrical stimulation is not strictly analogous to changes in hypothalamic temperature, and the reported responses may reflect the integrative rather than receptive function of the hypothalamus. For example, Wünnenberg and Brück (28) describe an afferent pathway (inhibitory to shivering) from the spinal cord to the posterior hypothalamus and Berzis and Hemingway (1-3) have reported an efferent pathway that carries signals from the posterior hypothalamus down the spinal cord to shivering effectors (i.e., skeletal muscle). Thus, the effects of electrical stimulation on shivering activity could represent direct activation of efferent pathways and as such would be independent of hypothalamic temperature. This interpretation is consistent with the finding that cold-exposed rats are still capable of shivering even when the POAH region is ablated (21).

On the basis of these considerations and the results of the present experiments, it would appear that in cold-exposed rats the increased temperature of the POAH may directly affect ST (e.g., via the feedback pathway shown as a dotted line in Fig. 5). The extent to which these data can be generalized to include other mammals is not clear since the necessary information (i.e., the effect of receptor warming on both ST and NST in cold-exposed animals) is not yet available. For example, although warming the hypothalamus of a dog maintained at an ambient temperature of 22 ± 0.5°C appeared to suppress shivering (14) no measure of NST was obtained.

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On the basis of these considerations and the results of the present experiments, it would appear that in cold-exposed rats the increased temperature of the POAH is directly involved in the regulation of NST but has no such direct action on shivering. That is, in these animals, thermal information from cutaneous and hypothalamic thermoreceptors does not appear to be integrated in the same fashion by neurocontrollers for shivering and nonshivering heat production. Thus, although species differences may exist, data currently available are consistent with the suggestion that warming the anterior hypothalamus while lowering ambient temperature depresses nonshivering thermogenesis while only indirectly affecting shivering.

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REFERENCES


