CNS regulation of body temperature during hibernation

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The body temperature (Tb) of hibernating golden-mantled ground squirrels, Citellus lateralis, passively follows ambient temperature (Ta) down to some critical temperature below which metabolic heat production increases and the differential between Tb and Ta increases. This paper demonstrates that such apparent thermoregulatory responses during hibernation are mediated by the central nervous system (CNS) regulator of Tb located in the preoptic nuclei and the hypothalamus (POH). By heating and cooling the POH of hibernating ground squirrels, it was possible to elicit changes in metabolic heat production which can be described by the equation: Rr - Ro = α(Thy - Tset); where Thy is POH temperature, Tset is the threshold POH temperature for activating the response in question, α is a proportionality constant, Rr is the basal rate of the response when Thy = Tset, and Rr is the response rate elicited by a Thy below Tset. For 10 response curves constructed for six animals, α averaged -4.46 × 10^-4 W g^-1 °C^-1 and ranged from -2.79 × 10^-4 to -6.48 × 10^-4 W g^-1 °C^-1. Tset averaged 6.3°C and ranged from 1.2 to 12.5°C. The Q10 for the increase in α from a Tset of 6°C to a Tset of 36°C (euthermia) is 2.5. It is hypothesized that the same populations of neurons are responsible for thermoregulation in euthermia and deep torpor. The evolution of an adequate α for driving thermoregulatory responses when Tset is very low during torpor may also have given rise to the extremely high α values measured on euthermic ground squirrels.

WHETHER THE CENTRAL NERVOUS SYSTEM of a hibernating mammal can regulate body temperature has long been debated in the literature. Much evidence indicates that torpid or hibernating mammals can activate various physiological mechanisms which might be considered thermoregulatory. Animals going into torpor may depart from a passive cooling curve with bursts of increased heat production (4, 9, 15, 18). Animals known as deep hibernators sometimes do not allow body temperature (Tb) to come into equilibrium with ambient temperature (Ta), but through bursts of increased heat production cause Tb to cycle over a range above what would be equilibrium Tb if metabolic rate were determined solely by tissue temperature and the activation energies of metabolic processes having normal temperature coefficients (3, 5, 8, 10, 15). Many animals which undergo shallow torpor resist a drop in Tb below a certain level, and as Tb falls below that level, the differential between Tb and Ta increases (13, 17, 10). Similarly, it has been shown for two species of bats in deep hibernation that although Tb passively equilibrates with Ta down to 5°C, below 5°C further decreases in Tb result in an increase in heart rate and an increased differential between Tb and Ta (15). The crucial questions still remain: are these potential thermoregulatory responses controlled by an active CNS regulator? And is that regulator generating a neural driving force proportional to an error signal derived by comparing some function of Tb to a reference? If the answer to these two questions is yes, that means there is CNS regulation of Tb in deep hibernation and we can then ask what are the characteristics of the regulator during torpor, and how does it compare with the euthermic regulator?

The body temperature regulator of euthermic hibernators resides in the preoptic and hypothalamic nuclei (POH), and its main feedback loop is its own temperature (Tb). This paper demonstrates that the CNS regulator of Tb during hibernation is also located in the POH (5). We have suggested for two species of bats in deep hibernation that although Tb passively equilibrates with Ta down to 5°C, below 5°C further decreases in Tb result in an increase in heart rate and an increased differential between Tb and Ta (15). The crucial questions still remain: are these potential thermoregulatory responses controlled by an active CNS regulator? And is that regulator generating a neural driving force proportional to an error signal derived by comparing some function of Tb to a reference? If the answer to these two questions is yes, that means there is CNS regulation of Tb in deep hibernation and we can then ask what are the characteristics of the regulator during torpor, and how does it compare with the euthermic regulator?

The POH of hibernating ground squirrels and marmots has been shown to be temperature sensitive and to respond to changes in its temperature by activating thermoregulatory mechanisms. Golden-mantled ground squirrels (Citellus lateralis) can be induced to arouse from deep torpor by direct POH cooling with implanted thermodes, and this arousal can be suppressed by heating the POH. Also, if the POH is gently heated, lowered ambient and body temperatures which normally induce arousals fail to do so (5). Similar results have been obtained by Lyman and O'Brien (10, 11) on C. lateralis, C. tridecemlineatus, and Mesocricetus brandti by heating and cooling the whole head region with a spoon-shaped external thermode. Mills and South (12) have directly cooled the POH of hibernating marmots through the use of chronically implanted thermodes and demonstrated that the hibernating marmot responds to POH cooling by increasing heart rate and heat production. Moreover, their observations suggest that the responses are proportional to the degree of cooling, but they do not present any quantitative data on thermoregulatory responses which would enable calculation of a proportionality constant.
The purpose of the study presented in this paper was to determine whether the POH of the hibernating ground squirrel acts as a proportional temperature regulator. If so, we wanted to measure the characteristics of the Tb regulator of the torpid mammal and compare them to the characteristics of the regulator during euthermia. It was hoped that such a study would add new insights to the process by which the mammalian thermostat is reversibly altered to permit the lowering of body temperature during torpor.

MATERIALS AND METHODS

Golden-mantled ground squirrels (Citellus lateralis) were trapped in the Sierra Nevada near Bishop, Calif. They were caged individually in a controlled temperature room and held at 5 ± 2°C (121:121). Each animal had a nest box, cotton nesting material, and food (sunflower seeds) and water ad libitum. Occasionally the animals were also given Purina laboratory chow, fruits, and vegetables.

The thermode assembly used to heat and cool the POH and the surgical procedures used in implanting the thermodes were as previously described (5). In some animals an 18-gauge polyvinyl tube closed at one end was positioned subcutaneously in the interscapular region, and the open end was attached to the thermode assembly. This tube served as a thermocouple reentrant tube for measuring an anterior body temperature.

The experimental apparatus has also been described previously (2). The experimental procedure was to transfer a hibernating animal from its nest box to a metabolism chamber in a controlled temperature box which had previously been equilibrated to the desired experimental temperature. Thermocouples were placed in the reentrant tubes and in some experiments a thermocouple was also inserted 3–4 cm into the rectum and taped to the base of the tail. Thermode perfusion lines were attached to the thermode assembly on the animal’s head. This procedure always induced at least a partial arousal. The animal was then allowed to come into equilibrium with ambient temperature which usually required several hours. POH temperature was manipulated by circulating water at controlled temperatures through the thermodes.

Metabolic rate was measured as oxygen consumption by pulling a steady flow of dried ambient air through the metabolism chamber after which it was redried and passed through a Beckman F3-M3 oxygen analyzer. The plumbing of the airflow system enabled periodic sampling of ambient air without interrupting the flow rate through the metabolism chamber. The output of the O₂ analyzer went to a Leeds & Northrup 12-point recording potentiometer giving a continuous record of ΔPO₂ between ambient air and effluent air from the metabolism chamber + 0.05 mmHg. Each day the O₂ analyzer was calibrated against dry atmospheric air at several subatmospheric pressures to determine the partial pressure of oxygen equivalent to the millivolt output of the analyzer. Using this factor and the flow rate (S/F) through the metabolism chamber and assuming an RQ of 0.71, the O₂ analyzer output was translated to watts. The average metabolic rate over any experimental period was determined by measuring the area under the O₂ consumption curve with a planimeter.

RESULTS

An experiment in which a hibernating golden-mantled ground squirrel was subjected to a very slowly decreasing ambient temperature is presented in Fig. 1. The temperature of the chamber was lowered from 2.0 to −1.8°C in steps of no more than 0.2°C over a period of 7 days. The temperatures of the animal’s POH, rectum, and subcutaneous interscapular area were continuously monitored. These three body temperatures remained tightly clustered and passively tracked Tₐ down to a Tₐ of 0.5°C. As Tₐ fell below 0.5°C, the body temperatures diverged and no longer passively followed Tₐ. The temperature of the interscapular area was consistently higher than the other body temperatures and was the first to begin increasing as Tₐ continued to decrease. It should be noted that the last data points recorded were those at the lowest ambient temperature, and at that time and for several hours thereafter, there was no indication of an arousal from deep torpor until the animal was physically disturbed by returning it to its home cage. These results demonstrate a thermoregulatory effort to hold some body temperature above some lower limit. The fact that the subcutaneous temperature in the interscapular area was consistently highest after the thermoregulatory response was initiated suggests the brown fat deposits in the thoracic cavity and the axillary regions were responsible for the thermogenesis. We cannot explain why the body temperatures increase below Tₐ = −1.0°C but can suggest two possibilities. As the difference between the regulated Tₐ and ambient temperature increases, there would be a greater amount of heat loss between site of thermogenesis (brown fat) and site of reception (POH);
and, therefore, a compensatory increase at and near the site of thermogenesis would be expected. This suggestion would be supported if \( T_{by} \) remained constant while the other body temperatures increased as \( T_b \) decreased. In the experiment \( T_{by} \) rose the least, but it did rise. The second possibility is an increasing input to the regulator from peripheral temperature receptors as \( T_b \) decreased. Evidence for such peripheral inputs to the regulator of the hibernating marmot has been described by Luecke and South (1) and to the regulator of the hibernating dormouse by Lyman and O'Brien (11).

The fact that the animal resists a lowering of \( T_b \) below a lower limit suggests that it would encounter metabolic and physiological malfunctions at lower body temperatures. The following observation lends credence to that suggestion. A hibernating golden-mantled ground squirrel was placed in the metabolism chamber at a \( T_b \) of \(-1.0°C\) and it maintained a rectal temperature of \(1.2°C\) by cyclical bursts of heat production similar to the pattern shown in Fig. 3. When POH was heated to \(4.5°C\) metabolic heat production dropped to basal levels also similar to the pattern shown in Fig. 3. POH was held at 4.0-4.5°C for 5 h while \( T_b \) was decreased to \(-2.5°C\), and during this time rectal temperature dropped to \(-0.5°C\). When the POH heating ceased, \( T_{by} \) also fell to \(-0.5°C\). Metabolic rate began to increase at this time and within 1 h was 2.5 times the level during POH heating, brown fat temperature increased to \(0.3°C\). This metabolic effort was not sustained and within another hour was back to basal levels, and body temperatures continued to fall. Respiration ceased at a rectal and hypothalamic temperature of \(-1.0°C\). During a 1-h-45-min period with no perceptible oxygen consumption, rectal temperature fell to \(-1.6°C\) and \( T_{by} \) fell to \(-1.8°C\). At this time the animal was taken from the chamber. No respiratory movements or heartbeat were detectable. The animal was slowly rewarmed, and its heart was stimulated with an intracardiac injection of epinephrine.

The animal recovered fully and subsequently hibernated and aroused spontaneously many times. This observation was not repeated, but it strongly suggests that the lowest body temperatures experienced by these hibernators are very close to the lower limit for cardiovascular and respiratory functions and the POH regulator is operating to hold \( T_b \) above that lower limit.

The following experiment was designed to determine whether apparent thermoregulatory activities of animals in deep hibernation such as shown in Fig. 1 are controlled by the same CNS mechanism which regulates \( T_b \) in the euthermic animal. We also wanted to determine whether the temperature of the preoptic and hypothalamic tissues serves as the main feedback loop to this regulator in deep hibernation as in euthermia. Therefore, we heated and cooled the POH of ground squirrels in deep hibernation at \( T_{by} \)'s close to their lower limit while monitoring metabolic heat production and various body temperatures. A portion of the record obtained in one such experiment is shown as Fig. 2. Ambient temperature was held at \(3.5°C\) throughout this experiment, and prior to the first manipulation of POH temperature (\( T_{by} \)) body temperature was steady at \(3.7°C\) and metabolic rate was steady at \(2.09 \times 10^{-4} \text{ W g}^{-1} \).

There had been no bursts of heat production or rises in \( T_{by} \) for the 16 h the animal had been in the chamber prior to this record. \( T_{by} \) was first cooled to 3.0, 2.5, and \(1.9°C\) with no response, but when it was cooled to \(1.6°C\), a rise in metabolic rate ensued. As can be seen from Fig. 2, greater decreases in \( T_{by} \) resulted in greater rises in metabolic rate. During periods of POH heating, metabolic rate returned to basal levels.

The last period of POH cooling in Fig. 2 is a prolonged one and shows that even though the induced increase in metabolic rate characteristically occurs in bursts, these bursts cycle if the \( T_{by} \) which induced the first burst is maintained. This cyclical pattern of increased heat production appears similar to that observed in animals hibernating at low ambient temperatures and not allowing their body temperatures to come into passive equilibrium with \( T_b \). We have reported this phenomenon before (5), and another example is given in Fig. 3. The fact that such naturally occurring cyclical bursts of heat production can be suppressed by heating the POH shows that they are under the control of the CNS regulator of body temperature.
Another feature apparent in Fig. 2 which is typical of all such experiments is that there is approximately a 10- to 15-min delay between the onset of POH cooling and the beginning of the increase in metabolic heat production. Since our experimental system for analysis of oxygen consumption has a lag time of less than 1 min to detecting any change in O2 concentration in the metabolism chamber, this delay is of biological origin.

The data from Fig. 2 were combined with additional data on the same animal under the same conditions on the following day and are presented graphically in Fig. 4. Each point represents the average metabolic rate over a period of 0.5 h commencing 10-15 min after a change in Thy or during a 0.5-h period when Thy was not manipulated. There is obviously a very close correlation between the data on the same animal under the same conditions on the following day and are presented graphically in Fig. 4. Each point represents the average metabolic rate over a 15-min delay between the onset of POH cooling and the beginning of the increase in metabolic heat production. Since our experimental system for analysis of oxygen consumption has a lag time of less than 1 min to detecting any change in O2 concentration in the metabolism chamber, this delay is of biological origin.

The resulting fluctuations in body temperature are also greater and Thy may overshoot Tset. When Thy does exceed Tset due to a burst of heat production, the metabolic rate falls to basal levels and the next burst of heat production does not occur until Thy again falls below Tset.

The experimental situation described above of an animal hibernating at a Ta lower than Tset provides additional evidence without artificial manipulations of Thy that the thermoregulatory heat production response of animals in deep torpor is controlled by a proportional CNS regulator (Fig. 5). The average metabolic rate from initiation of burst to peak of burst is plotted in Fig. 5 as a function of Thy. 15 min prior to the initiation of the burst (solid points). The resulting curve is very similar to the one in Fig. 3 obtained by heating and cooling the POH with thermodes. The open circles in Fig. 5 represent average metabolic rates measured over periods of time when Thy was controlled with thermodes. Some of the data points above Tset were also obtained by manipulating Thy with thermodes. Clearly the data obtained by manipulating Thy corresponds perfectly with data obtained during naturally occurring fluctuations of Thy. The basal data points above Tset in Fig. 5 were obtained during periods when Thy was high following a burst of heat production and also during periods of POH heating. The a value calculated from the data in Fig. 5 is \(-4.32 \times 10^{-4}\) W g\(^{-1}\) °C\(^{-1}\) and Tset is 8.3°C.

Ten curves such as those in Figs. 4 and 5 relating metabolic heat production to POH temperature were obtained from 6 C. lateralis (Table 1). The threshold POH temperature for the response (Tset) varies greatly between animals and for the same animal on different days, but on 1 day or even for several days in succession, Tset may remain very constant unless an arousal from torpor is initiated. It is

![Graph](image-url)
interesting to note that the higher Tset's seem to occur later in the hibernation season, however, this may be a spurious correlation, since it is a comparison of different individuals. The proportionality constants (a) for the metabolic heat production response to POH cooling average $4.46 \times 10^{-4}$ W g$^{-1}$ C$^{-1}$ and range from $-2.79 \times 10^{-4}$ to $-6.48 \times 10^{-4}$ W g$^{-1}$ C$^{-1}$ and in all cases the correlation coefficients are significant at the $P < .05$ level. Unfortunately, the data are not sufficient to test for any correlations between $\alpha$ and Tset which will be discussed below.

A common response to POH cooling which is not shown in the figures or in the table is partial or complete arousal from torpor. As we cooled the POH farther and farther below Tset, the arousal response would eventually be elicited in most experiments. A full arousal results in a return to the euthermic body temperature, but a partial arousal is not so clearly distinguishable from proportional thermoregulatory responses such as shown in Fig. 2. We defined as partial arousals any responses which fell very much to the right of the regression lines of the response curves and which were associated with a changed Tset. Such responses were not included in calculating the proportionality constants in Table 1.

**DISCUSSION**

These results clearly show that over a small range of POH temperatures, the POH of hibernating golden-mantled squirrels is capable of activating metabolic heat production responses which are proportional to the difference between POH temperature and some threshold temperature (Tset). Such a capability is clearly an adaptive advantage for the animal. The hibernating animal resists a fall in its body temperature below a lower limit which may change during the hibernation season (3, 17). If Tset fell much below that lower limit, the animal might suffer metabolic and physiological malfunctions and would not be able to arouse spontaneously. Since temperature fluctuations in a well insulated, subterranean nest will be small and slow, it is reasonable that the hibernator's first line of defense to protect itself from dangerously low Tset's should be a proportional thermogenesis and not the costly metabolic response of full arousal.

Two previous studies (3, 5), concluded that the golden-mantled ground squirrel does not show proportional regulation of body temperature in deep hibernation; we can resolve the discrepancies between the conclusions of those reports and this one. Hammel et al. (3) based their conclusion on the observation that the Tset's of their experimental animals passively equilibrated with Tset's between 2.3 and 13.0°C and metabolic rate was directly proportional to Tset with a Q10 of 2.4. Since the lowest Tset in their study was 3°C, it is possible that Tset was never below Tset in the experiments from which they calculated the Q10 of metabolic rate. At Tset's below 2.3°C they observed bursts of heat production which sometimes cycled on and off and frequently led to full arousals. They termed these bursts partial arousals and interpreted them as being due to activation and inactivation of the CNS regulator. In the context of the present study, many of these bursts may have been proportional responses to POH temperatures below Tset.

Heller and Hammel (5) heated and cooled the POH of hibernating golden-mantled squirrels, and over a broad range of ambient and body temperatures noted either no metabolic response to POH cooling or bursts of heat production. Since these bursts resulted in full arousal if POH cooling was maintained and since in several cases it was demonstrated that Tset had increased following the response, these bursts were considered, and may well have been, partial arousals. It is likely that we did not cool POH to Tset in most cases, and since we used rather large step function drops in Tset, when we did cool POH below Tset, it created a sufficiently large error signal in most cases to induce a partial arousal. Some of the responses we observed probably were proportional responses, but in general we missed the rather small range of POH temperatures below Tset over which proportional thermoregulatory responses are elicited from the hibernating ground squirrel.

The present study confirms our earlier report (5) that cooling the POH of a hibernating ground squirrel can induce a rise in Tset resulting in a partial or full arousal from torpor. We now add to that conclusion that the hibernating ground squirrel is capable of a proportional thermoregulatory response to POH cooling without a change in Tset. There are probably two separate mechanisms involved.

Our working hypothesis in this research is Hammel's model of the CNS regulator of body temperature (1, 2, 4). The basic components of this model are two populations of POH neurons which have different Q10's (Fig. 6). The high Q10 population activates mechanisms of heat loss and inhibits mechanisms of heat production-conservation. The low Q10 population activates mechanisms of heat production-conservation and inhibits mechanisms of heat loss. The intersection of the firing rate vs. temperature curves of these two populations of neurons therefore creates a set point for body temperature, for it is the only POH temperature which does not generate a net driving force for heat
Hammel's neuronal model for CNS regulator of body temperature and predictions based on this model for how characteristics of regulator would change if \( T_{t} \) could be regulated at any level between 0 and 10°C. Heat loss and heat production represent target organs for affecting rates of heat loss from the body and rates of heat production by thermogenic tissue. Single neurons in model represent cell populations; 4 basic types are indicated in the POH. Neurons 1 and 2 differ in their degree of temperature dependence and both facilitate and inhibit neurons like 3 and 4 which have no spontaneous activity or temperature dependence. Neurons 1 and 2 receive facilitative and inhibitory inputs from a variety of sources. Graph at bottom of figure shows hypothetical firing rates of neurons 1 and 2 as a function of POH temperature for the dog and the ground squirrel. In both animals when firing rates of neurons 1 and 2 are equal, neurons 3 and 4 are inhibited more than facilitated and no net driving force is generated for heat loss or heat production mechanisms. If POH is heated, neuron 3 will be more facilitated than inhibited and will drive heat loss.

Thus, threshold temperature \( (T_{set}) \) for a thermoregulatory response depends on relationship between neurons 1 and 2, and this can be changed by inputs to regulator mentioned above. For example, entrance into hibernation may be achieved by decreased input from BSRF (brainstem reticular formation) to neuron 2. As activity of neuron 2 decreased (dashed lines on ground squirrel curve), \( T_{set} \) would decrease. In both curves for ground squirrel and for dog, \( Q_{10} \) of neuron 2 is 1 and the \( Q_{10} \) of neuron 1 is 2.5, but ground squirrel curves are shifted to left. At any \( T_{set} \), proportionality constant for a thermoregulatory response \( (\alpha) \) is a function of difference in slopes of firing rate versus temperature curves of neurons 1 and 2; hence at any \( T_{set} \), regulator of ground squirrel would produce a greater \( \alpha \) than regulator of the dog. This may be an adaptation for thermoregulation during hibernation. Numbers labeled "a" represent measured \( \alpha \) values in cal g\(^{-1}\) min\(^{-1}\) °C\(^{-1}\). Dashed line indicating a feedback loop from neuron 4 to BSRF suggests existence of a positive feedback mechanism for elevating activity of neuron 2 and hence \( T_{set} \) during an arousal. (Figure modified from ref. 5.)

In terms of this model let us make the simplifying assumptions that the low \( Q_{10} \) neurons have a \( Q_{10} \) of 1 and the entrance into hibernation involves an inhibition of these neurons so that the set point for body temperature decreases. Let us also assume that the \( Q_{10} \) of the high \( Q_{10} \) population is a biologically reasonable value somewhere between 2 and 3, and the \( Q_{t} \)'s of both populations of neurons do not change over the range of body temperatures experienced by the hibernator. Then, as \( T_{set} \) declines when an animal drops into hibernation, the proportionality constants for thermoregulatory responses should also fall with a \( Q_{t} \) between 2 and 3.

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The proportionality constant \( (\alpha) \) for any thermoregulatory response to changes in POH temperature will be a function of the difference in slopes of the firing rate-temperature curves of these two populations. Many factors may change the set point, presumably by inhibiting or facilitating one or both of these two populations of neurons and thereby shifting the point of intersection of their firing rate versus temperature curves (1, 4).
one temperature, but their $Q_{10}$ remains the same. Hence, the difference between the slopes of the two $Q_{10}$ curves is increased over any temperature range (Fig. 6). The corollary of such an adaptation for increasing $\alpha$ at low body temperatures would be that $\alpha$ during euthermia would also be proportionally higher.

This simple hypothesis describing a possible mechanism for decreasing $T_{set}$ during hibernation while still allowing for thermoregulation in deep torpor would gain credence if additional data on individual animals during different bouts of torpor show that $\alpha$ is related to $T_{set}$ by a $Q_{10}$ function between 2 and 3. If it is not, we may have to postulate a third population of neurons responsible for creating $T_{set}$ and driving metabolic heat production response during deep torpor. In this regard, it is tempting to think of central units similar to the peripheral units found in the facial skin of the hamster which increase their firing rates as temperature decreases, reaching a maximum firing rate at $4^\circ C$ (14).

Our view of the CNS regulator of body temperature of hibernators will become less speculative and more accurate as we obtain more information from C. lateralis and other small mammals which display various forms of torpor as well as on other species of small mammals which are obligate homeotherms. From a comparative approach we hope to be able to explore a variety of hypotheses in an effort to understand the thermoregulatory abilities of hibernating ground squirrels and the significance of the extremely high $\alpha$ values of euthermic ground squirrels.

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