Central Nervous Regulation of Body Temperature During Sleep

Abstract. *The relationship between hypothalamic temperature and metabolic heat production was measured during wakefulness, slow-wave sleep, and paradoxical sleep in unrestrained kangaroo rats (*Dipodomys*). Hypothalamic temperature was manipulated with chronically implanted, water-perfused thermodes while cortical electroencephalogram, electromyogram, metabolic rate, and body movement were continuously recorded. During slow-wave sleep, in comparison to wakefulness, there is a lowered threshold hypothalamic temperature for the metabolic heat production response and a lowered proportionality constant relating rate of metabolic heat production to hypothalamic temperature. During paradoxical sleep no increase in metabolic heat production could be elicited by lowering hypothalamic temperature, which indicates that the thermoregulatory system is inoperative. These results provide a basis for explaining the changes in various body temperatures, metabolic rate, and other thermoregulatory responses during sleep in a variety of mammals.*

A diurnal rhythm of body temperature (T_b) in man and other mammals has been well documented (*1*). This diurnal rhythm exists independently of sleep (*2*), but fluctuations of T_b specifically associated with sleep also occur (*3, 4*). Many of these changes in T_b are associated with specific sleep states and can be attributed to alterations in an important thermoregulatory effector mechanism, peripheral vasomotor tone (*4, 5*). The activities of other thermoregulatory effector mechanisms also undergo state-dependent changes. At neutral to warm ambient temperature (T_a) the sweat rate of man increases at the onset of slow-wave sleep (SWS) but is severely depressed during paradoxical sleep (PS) (*6*). The rate of metabolic heat production of man falls at the onset of SWS (*7*). Shivering, panting, and sweating responses to low and to high T_a occur during SWS, but they cease and are not seen during PS (*8*). Although these and other observations on a variety of species indicate that the activities of thermoregulatory effector organs change as a function of state of arousal, little is known about the associated changes occurring in the central thermoregulatory system. We present here the first quantitative comparison of the characteristics of the central nervous system (CNS) regulator of T_b during SWS, PS, and wakefulness.

The CNS regulator of T_b resides in the hypothalamus, and the temperature of the hypothalamus (T_{hy}) is a major feedback signal to the regulator (*9*). In some species of small mammals, including the kangaroo rat *Dipodomys ingens*, T_{hy} is the dominant feedback signal (*10*). In these species the relative unimportance of other feedback loops to the regulator makes it possible to approximate an "open loop" condition and take control of the system by using thermodes or other devices to manipulate T_{hy}. We can then describe the characteristics of the regulator by measuring the rate of some thermoregulatory response as a function of T_{hy}. The absence of extrahypothalamic feedback in addition to the ease with which kangaroo rats adjusted to and slept under experimental conditions made it possible for us to investigate the changes occurring in the thermoregulatory system during sleep.

Thermode assemblies were implanted in kangaroo rats (*Dipodomys*) as described (*10*) so that a pair of water-perfused thermodes with an intertip distance of 3 mm straddled the hypothalamus at the level of the anterior commissure and the optic chiasm. A thermocouple reentrant tube was positioned 0.5 mm off midline 1 mm anterior to the thermodes. Position of the thermodes and reentry tube was recorded postoperatively with an x-ray photograph. The confirmation of correct placement was obtained histologically at the end of the experiments. Stainless steel screws anchoring the thermode assembly to the skull served as cortical electroencephalograph (EEG) electrodes. Electromyograph (EMG) electrodes were inserted subdermally over the dorsal neck muscles. Gold contacts from all leads were cemented around the thermode assembly.

The care of the animals and the experimental apparatus were as described (*10*). The small volume of the metabolism chamber and its high ventilation rate resulted in a lag time of less than 35 seconds for the detection of changes in metabolic rate. Experiments were always conducted during the animals' inactive periods and at a thermoneutral temperature of 30°C, which maximized the occurrence of sleep (*10, 11*). Animals were allowed a 1.0- to 1.5-hour acclimation period after being placed in the metabolism chamber and attached to cables, thermocouple, and water-perfusion lines before data were collected. During an experiment we continuously recorded rates of O_2 consumption or CO_2 production (or both), metabolism chamber air and wall temperatures, perfusant water temperature, T_{hy}, frontal-occipital EEG, EMG, and gross body movements (*12*). Behavioral observations were made through the Plexiglas top of the metabolism chamber.

Changes in EEG, EMG, and respiratory rhythm during sleep (Fig. 1) were similar to those reported for other spe-

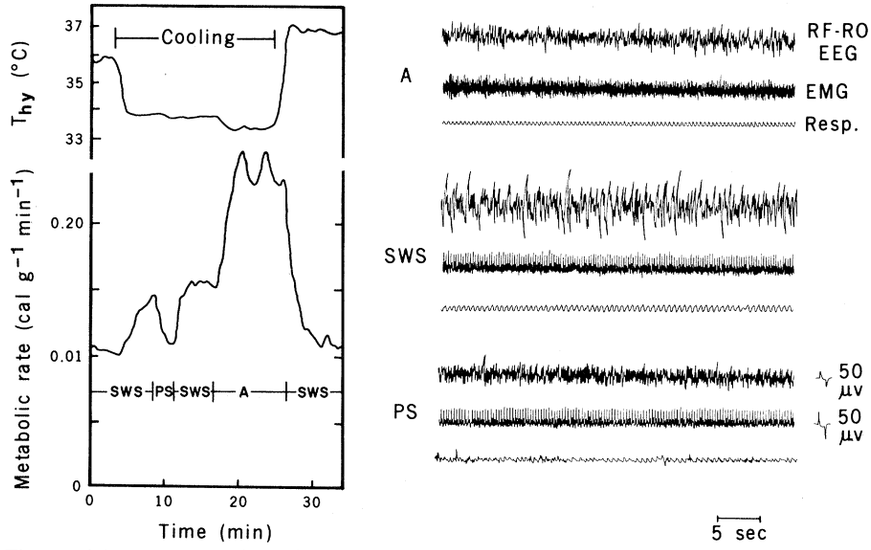

Fig. 1. (Right) Representative polygraph tracings obtained from one animal, typical of the recordings obtained from all subjects in this study. The respiratory movements record (Resp.) was obtained from a gross body movement detector, which also picked up phasic activity of skeletal muscles or "twitches" during paradoxical sleep (PS); A, awake; SWS slow-wave sleep. (Left) A typical recording of metabolic rate and hypothalamic temperature obtained during an experiment. Sleep states were defined by simultaneous polygraph recordings and superimposed on the figure. During SWS but not during PS the animals responded to hypothalamic cooling with an elevated metabolic rate. The response to hypothalamic cooling was much greater during wakefulness than during SWS.

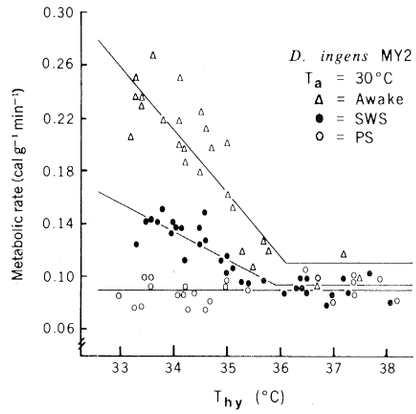

Fig. 2. Relationship between metabolic rate and hypothalamic temperature (T_{hy}) during the three states of arousal for one kangaroo rat. Each symbol represents the average metabolic rate during an uninterrupted period of wakefulness, SWS, or PS, with or without manipulation of T_{hy}. The slopes of these curves are presented in Table 1.

cies (*13*). More than 270 epochs of wakefulness, SWS, and PS with and without manipulations of T_{hy} were analyzed. In some cases T_{hy} was manipulated during a continuous epoch of wakefulness, SWS, or PS, but in other cases sleep states changed during a period of T_{hy} manipulation. In the example presented in Fig. 1 the initial lowering of T_{hy} was made during an epoch of SWS and elicited an immediate increase in metabolic rate. Five minutes after the beginning of this hypothalamic cooling the animal entered a 3.5-minute epoch of PS during which metabolic rate returned to a basal level, even though hypothalamic cooling was maintained. The animal returned to SWS after this bout of PS, and the metabolic rate returned to an elevated level similar to the initial response. At 17 minutes on this record T_{hy} was lowered an additional 0.5°C. The animal aroused and the metabolic rate rose rapidly to a much higher response level. When the manipulation of T_{hy} was terminated, the animal returned to SWS and metabolic rate returned to a basal level.

All of the responses of one animal to manipulation of T_{hy} during SWS, PS, and wakefulness are shown in Fig. 2. Metabolic rate during PS was independent of T_{hy} and averaged 0.088 cal g^{-1} min^{-1}. During wakefulness and SWS there appeared to be a threshold T_{hy} below which metabolic rate increased as T_{hy} decreased. This threshold was estimated, data points above it were averaged, data points below it were subjected to linear regression analysis, and the intersection of the regression line with the average was then taken as the actual T_{hy} threshold (T_{set}) for the metabolic heat production response. This threshold was slightly lower during SWS than during wakefulness. The major difference between SWS and wakefulness was, however, the lower slope of the response curve during SWS. This slope or proportionality constant, termed α_{MHP} by convention, was -0.049 cal g^{-1} min^{-1} $°C^{-1}$ during wakefulness and -0.021 cal g^{-1} min^{-1} $°C^{-1}$ during SWS.

The data obtained from three animals are summarized in Table 1. In each animal the threshold T_{hy} for the metabolic heat production response is slightly lower and the α_{MHP} is dramatically lower during SWS than during wakefulness. The absence of hypothalamic thermosensitivity during PS supports our earlier conclusions based on behavioral criteria for PS (*10*). In no instance did a lowering of T_{hy} elicit an increased rate of metabolism during PS, even though T_{hy} was cooled at least 4°C below normal in each animal. In addition to the data for three animals in Table 1, data from two other kangaroo rats also showed absence of hypothalamic thermosensitivity during PS (*14*). The results of these experiments indicate to us that the thermoregulatory system is functioning at a reduced level during SWS in comparison to wakefulness but that it is inactivated during PS.

A lack of hypothalamic thermosensitivity during PS has also been reported by Parmeggiani *et al.* (*15*), who used diathermy to raise T_{hy} of cats during SWS and PS. They described distinct T_{hy} thresholds for thermal polypnea and panting during SWS but not during PS, and concluded that the elevation of the threshold T_{hy} for these responses was "practically infinite" during PS. However, we believe that their results, like ours, indicate an inactivation and not a resetting of the regulator of T_b during PS. The alternative explanation of both sets of results would involve extreme rises of thresholds for heat loss responses concomitant with extreme falls of thresholds for heat production or conservation responses during PS. This is a difficult explanation to accept, since studies of conditions that alter T_{hy} thresholds for thermoregulatory responses show that a stimulus or condition that induces a rise in the T_{hy} threshold for a heat loss response simultaneously induces rises in the T_{hy} thresholds for heat production or conservation responses (*16*). Similarly, a stimulus that reduces the threshold for one response does so for all responses.

The downward resetting of the CNS regulator of T_b during SWS contributes support to the hypothesis that a primal and primary function of SWS is energy conservation (*17*). Especially for a small endotherm, a lowering of T_b during obligate periods of inactivity could result in significant decreases in metabolic energy expenditure. Mammals that hibernate or even enter shallow torpor incur enormous reductions in metabolic rate by lowering regulated T_b (*18*). Recent studies on ground squirrels entering hibernation have demonstrated that the CNS regulator of T_b is not turned off, but is progressively and slowly turned down during the entrance (*19*). Does this similarity in thermoregulatory changes between kangaroo rats during SWS and ground squirrels entering hibernation represent a functional homology? An affirmative answer to this question is suggested by electrographic studies of hibernators which demonstrate that they enter hibernation predominantly through SWS (*20*).

An inactivation of the thermoregulatory system during PS may explain the conflicting reports about changes in brain, skin, and other T_b during PS (*4*, *5*). It has been convincingly argued that

Table 1. Hypothalamic temperature thresholds (T_{set}) and proportionality constants (α) for the metabolic heat production response at T_a of 30°C during wakefulness (A), slow-wave sleep (SWS), and paradoxical sleep (PS) in three kangaroo rats; α is expressed in cal g^{-1} min^{-1} $°C^{-1}$; N is the number of observations; r^2 is the coefficient of determination for α; and P is the probability that α is not significantly different from zero.

Sleep status	T_{set}	α	N	r^2	P	α_A/α_{SWS}
		Dipodomys ingens MY2				
A	36.1	−0.049	23	.72	<.001	
SWS	35.9	−0.021	20	.56	<.02	2.4
PS		+0.0021	24	.16	>.10	
		Dipodomys ingens S4				
A	35.7	−0.069	19	.59	<.01	
SWS	35.2	−0.042	14	.68	<.01	1.6
PS		−0.0004	22	.01	>.10	
		Dipodomys heermanni MY751				
A	36.3	−0.045	14	.79	<.001	
SWS	36.1	−0.026	19	.61	<.01	1.8
PS		+0.001	17	.01	>.10	

changes in peripheral vasomotor activity may be primary causes of the various temperature changes observed during PS (*4*, *5*). The CNS regulator of T_b has a strong influence on peripheral vasomotor activity. Releasing peripheral vasomotor activity from thermoregulatory control during PS should result in changes in skin temperature, hence, changes in heat exchange between animal and environment and changes in core T_b's. The direction of these changes will depend on whether the peripheral vascular beds were dilated or constricted relative to the thermoneutral condition before the onset of PS.

The fact that proportional regulation of T_b is retained during SWS but not during PS offers an adaptive explanation for the relationship between ambient temperature and proportion of time spent in different sleep states observed in cats and rats. At low ambient temperatures the animals spend a greater proportion of their total sleep time in SWS and a lesser proportion in PS than at thermoneutral temperatures (*11*). The relative increase in SWS at low ambient temperatures enables the maintenance of active thermoregulatory defenses against the cold without drastically curtailing total sleep time.

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Dopaminergic Agents: Influence on Serotonin in the Molluscan Nervous System

Abstract. *Treatment of the mussel* Mytilus edulis *with 6-hydroxydopamine or with α-methyl-p-tyrosine decreased dopamine and increased serotonin in the nervous system. Treatment with dopamine decreased serotonin concentrations and prevented the effect of 6-hydroxydopamine. The serotonin concentration appears to be determined in part by the concentration of dopamine.*

The regulation of the rate of synthesis and turnover of neurotransmitters is of great interest (*1*). Regarding the synthesis of monoamines in the mammalian brain, it was observed that 5-hydroxytryptophan, a precursor of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), decreased the rate of synthesis of norepinephrine (NE) (*2*), whereas norepinephrine decreased the rate of synthesis of 5-HT (*3*). It was suggested that, in situations where dopamine (DA)–containing and 5-HT–containing neurons interact, each type might inhibit the synthesis of the other's transmitter (*3*). The central nervous systems of molluscs have neurons that contain predominantly dopamine (DA) or 5-HT, but the possibility that the rates of synthesis or neuronal content of DA and 5-HT might be interdependent in these animals does not seem to have been studied. In various species the DA and 5-HT content of different ganglia or neurons is influenced by several drugs, such as 6-hydroxydopamine (6-OHDA), dihydroxyphenylalanine, and α-methyl-*p*-tyrosine (α-M*p*T) (*4*, *5*). We have altered, by selective drug treatments, the DA content of neurons in a representative mollusc, *Mytilus edulis*, and have observed changes in the concentrations of 5-HT and DA. The 5-HT content of the ganglia appears to be determined, at least in part, by the concentration of DA.

The marine mussel *Mytilus edulis* was obtained from Woods Hole (Northeast Marine Specimen Company), or collected locally from Long Island Sound and kept in artificial seawater (ASW) at *p*H 7.0 to 7.2 and at 18° to 20°C. The drugs we used were DA, 6-OHDA, α-M*p*T, and 5-HT (all from Sigma Chemical Company). Each drug (1 mg) was dissolved in ASW (1 ml) containing ascorbic acid (0.1 percent). We injected 10 μl of fresh solution into the posterior adductor muscle through a small notch cut in the posterior dorsal rim of the shell near the posterior adductor. Control animals were injected with 10 μl of ASW with ascorbic acid. Each experiment was performed on mussels obtained from the same place at the same time and having the same shell length. Experiments lasted 6 days, each mussel receiving three injections given on alternate days. After treatment, animals were opened and tissue was dissected out; this was either extracted for chemical analysis or freeze-dried for histofluorescence studies. In initial experiments with 6-OHDA we used the same procedure except that we added the drug (4.8 mg) to the ASW (300 ml) bathing an individual mussel. The changes in fluorescence (*6*) were similar to those we report here.

For the histofluorescence studies, excised tissue was freeze-dried and treated with hot paraformaldehyde gas as described by Falck and co-workers (*7*). We used a Reichert Zetopan fluorescence mi-