The Journal of Experimental Biology **198**, 931–937 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

PARASYMPATHETIC INFLUENCE ON HEART RATE IN EUTHERMIC AND HIBERNATING GROUND SQUIRRELS

MICHAEL B. HARRIS AND WILLIAM K. MILSOM

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, Canada V6T 1Z4

Accepted 9 November 1994

Summary

The relative role of the parasympathetic nervous system during deep hibernation is enigmatic. Conflicting hypotheses exist, and both sides draw support from investigations of vagal influence on the heart. Recent studies have shown cardiac chronotropic and inotropic effects of parasympathetic stimulation and inhibition in isolated hearts and anesthetized animals at hibernating body temperatures. No studies, however, have demonstrated such occurrences in undisturbed deeply hibernating animals. The present study documents respiratory-related alterations in heart rate during euthermia and hibernation at ambient temperatures of 15, 10 and 5 °C mediated by parasympathetic influence.

During quiet wakefulness, euthermic squirrels breathed continuously and exhibited a 29 % acceleration in heart rate during inspiration. During deep undisturbed hibernation, at 15, 10 and 5 °C ambient temperature, animals exhibited an episodic breathing pattern and body temperatures were slightly above ambient temperature. At each temperature, heart rate during the respiratory episode was greater than that during the apnea. The magnitude of this ventilatory tachycardia decreased with ambient temperature, being 108 % at 15 °C, 32 % at 10 °C and 11.5 % at 5 °C. Animals exposed to 3 % CO_2 at 5 °C, which significantly increased ventilation, still exhibited an 11.7 % increase in heart rate during breathing. Thus, the magnitude of the ventilation tachycardia was independent of the level of ventilation, at least over the range studied. Inhibition of vagus nerve conduction at 5 °C was achieved using localized nerve block. This led to an increase in apneic heart rate and abolished the ventilatory tachycardia. The results of this study indicate that vagal projections are involved in cardiorespiratory control during deep hibernation, i.e. that parasympathetic tone is still present and is involved in homeostatic regulation during hibernation.

Key words: heart rate, ventilatory tachycardia, apneic bradycardia, vagus, parasympathetic control, hibernation, ground squirrel, *Spermophilus lateralis*.

Introduction

The degree to which the parasympathetic nervous system is effective during deep hibernation and arousal from hibernation is a contested issue. One theory asserts that deep hibernation in the ground squirrel *Spermophilus* (*Citellus*) *lateralis* is maintained through active parasympathetic suppression of sympathetic tone. Removal of parasympathetic tone or sympathetic activation leads to arousal (Twente and Twente, 1978). A contrasting theory argues that parasympathetic influence decreases relative to sympathetic influence as animals enter the hibernating state. Autonomic control during hibernation is thus due predominantly, if not exclusively, to sympathetic regulation, and deep hibernation is maintained and arousal initiated primarily by changes in thermoregulatory control (Lyman and O'Brien, 1963). While the latter view predominates (Lyman, 1982; Burlington and Milsom, 1989), both sides of this debate have drawn support from early investigations of parasympathetic vagal influences on heart rate during hibernation (for a review, see Lyman, 1982).

For parasympathetic influence to change during hibernation, alterations must occur in any or all of the following: (i) central motor output, (ii) nerve conduction, (iii) transmitter release and (iv) target tissue sensitivity. Reductions in all these processes are imaginable, simply as a result of the reduction of body temperature from 37 °C, typical of euthermia, to 7 °C, typical of deep hibernation at a 5 °C ambient temperature. For the dominant view of autonomic control during hibernation to hold, however, some of these changes must exceed those seen for sympathetic influences.

Recently, O'Shea and Evans (1985) observed decreases in the contractile force of isolated bat ventricles at 7 °C in response to intermural nerve stimulation and exogenous acetylcholine (ACh). This response was blocked by the muscarinic antagonist hyoscine. From these observations,

O'Shea (1987) proposed that low body temperatures did not compromise muscarinic receptor function. Also, Milsom *et al.* (1993) recently determined that, in anesthetized hibernating *S. lateralis* at 7 °C, electrical stimulation following mechanical vagotomy resulted in a reduction in heart rate. Thus, the reduction in body temperature associated with hibernation did not block vagal conduction, ACh release from nerve terminals or the ability of the heart to react to ACh. The authors noted, however, that the amount of electrical stimulation necessary to elicit a change in heart rate was greater during hibernation than during euthermia, indicating that the vagal influence was reduced, but this reduction appeared to be in proportion to the decreased body temperature.

The remaining gaps in our understanding of the autonomic control of heart rate during hibernation are the effects of hibernation on parasympathetic motor output and a comparison of how the suite of changes in parasympathetic control relate to those that occur in sympathetic control. The current study addresses the first of these questions by examining the vagal influence on heart rate associated with the episodic breathing pattern typical of golden-mantled ground squirrels during hibernation. This study examines the hypothesis that there is parasympathetic motor output during deep hibernation and that this parasympathetic tone has a physiological influence on heart rate. Specifically, we propose that vagal tone is present and modulated by respiration, resulting in a pattern of ventilatory tachycardia and apneic bradycardia during episodic breathing.

Materials and methods

Adult golden-mantled ground squirrels [*Spermophilus lateralis* (Say)] were housed individually in a sound-insulated, climate-controlled chamber at constant ambient temperature (T_a of 20±1 °C) and photoperiod (12 h:12 h light:dark, lights on at 06:00 h). Squirrels had access to laboratory chow and water *ad libitum* supplemented intermittently with sunflower seeds. In late November, squirrels were induced to hibernate by a gradual reduction of chamber temperature (to a T_a of 5±1 °C) and photoperiod (2 h:22 h light:dark, lights on at 10:00 h). Most animals entered hibernation within 2 weeks of exposure to these 'winter' environmental conditions.

Beginning in mid-February, individual hibernating animals were removed from the environment chamber and kept for 3 days at room temperature and uncontrolled photoperiod. Animals then underwent surgery for the implantation of recording electrodes and infusion cannulae.

Surgery

Animals were anesthetized with intraperitoneal injections of sodium pentobarbital (Somnotol, 65 mg ml^{-1} solution; 45–65 mg kg^{-1}). Four cranial electroencephalographic (EEG), two electrocardiographic (ECG), two electromyographic (EMG) and two respiratory impedance electrodes were implanted in the skull, rib-cage, shoulder musculature and abdominal wall, respectively. Electrode wires were run subcutaneously to a 10-pin connector cemented to the skull with dental epoxy resin. In animals to be used during the vagal blockade study, a second incision was made in the ventral neck and blunt dissection was used to expose and isolate the left and right cervical vagi. The right vagus was bathed in 2 % lidocaine hydrochloride (Xylocaine, Astra Pharmaceutical), severed and a 5 mm section was removed. The left vagus was placed within an 'infusion cuff', consisting of a 4 mm length of 2 mm i.d. silicone tubing slit down one side, with a length of 0.5 mm i.d. silicone cannula fastened in the middle of its inside edge. Two loops of suture closed the cuff about the nerve, and both ends were plugged with sterile petroleum jelly. Fluid fed down the cannula filled the cuff and bathed the isolated section of nerve. Subsequent infusions renewed the fluid within the cuff while the overflow seeped out of the slit edge of the cuff. Pilot investigations with anesthetized rats and ground squirrels indicated that a 0.04 ml Xylocaine infusion bathed the nerve and resulted in a complete nerve block within 30 s.

Care was taken to minimize stress on the nerve. The cuff was secured in position and the cannula fed subcutaneously over the shoulder to a connector mounted to the skull. All surgical procedures were carried out with full aseptic precautions. The incisions were closed and the animal allowed to recover for 3–5 days.

Following recovery, animals were either maintained at room temperature or moved to a second sound-insulated, climate-controlled chamber at constant ambient temperature (T_a of 5±1 °C) and reduced photoperiod (2 h:22 h light:dark, lights on at 10:00 h). Most animals at 5 °C re-entered hibernation within 2 weeks.

Experimental protocol

Initial investigations

Cardiorespiratory coordination and heart rate were observed in five animals during non-sleeping euthermia at an ambient temperature of approximately 22 °C and in six animals during hibernation at ambient temperatures of 15, 10 and 5 °C. Individual animals were transferred to an acrylic box (10 cm×10 cm×10 cm) within a laboratory controlled-environment chamber. Each box was supplied with air at a flow rate of 500 ml min^{-1}. The EEG, EMG and ECG were monitored using Gould universal amplifiers, and respiratory movements were monitored using an impedance converter (Biocom Inc., model 991) and Gould d.c. amplifier. All signals were recorded continuously on a Gould six-channel polygraphic recorder. Data were analyzed to determine euthermic heart rates during inspiration and during the post-expiratory non-ventilatory period (NVP) and mean heart rates in hibernation during breathing episodes (eupnea) and pauses between episodes (apnea). Inspiratory and NVP heart rates and eupneic and apneic heart rates were compared using a Student's *t*-test (P<0.05, SigmaStat, Jandel Scientific) to identify respiratory-related cardio-acceleration. Differences in heart rates between eupnea and apnea, or between inspiration and NVP, were expressed as a proportion of apneic or NVP

heart rate, resulting in a relative measure of the respiratory-related tachycardia during both euthermia and hibernation at 15, 10 and 5 °C.

Vagal blockade studies

Vagal blockade studies were performed on four animals during hibernation at an ambient temperature of 5 °C. Individual animals were again transferred to a box within a laboratory controlled-environment chamber and supplied with air at a flow rate of 500 ml min^{-1}. The EEG, EMG and ECG were monitored using Grass a.c. amplifiers, and respiratory movements were monitored using an impedance converter (Biocom Inc., model 991) and a Grass d.c. amplifier. All signals were recorded continuously by a Grass polygraphic recorder and a computerized data-acquisition system (AT-CODAS, DataQ Instruments Inc.) sampling at 40 Hz.

Following a 2–3 h acclimation period, experimental treatments were begun. Animals were exposed, for approximately 1 h each, to normocarbia (room air, 0.04 % CO_2) and hypercarbia (3 % CO_2 in air) before and after 0.04 ml infusions of 0.9 % NaCl (sham condition) and 2 % lidocaine hydrochloride (vagal blockade) into the cuff. The degree of vagal blockade was assessed by subsequent lidocaine infusion following the observation period.

Rectal temperatures were measured at the beginning of each test, but were not monitored continuously during these experiments. A previous study (Harris and Milsom, 1994*a*) demonstrated that animals in a similar experimental apparatus maintained stable body temperatures of approximately 7 °C until arousal from hibernation was initiated. Animals maintained deep hibernation and exhibited no signs of arousal during the present study. Instantaneous heart rate was calculated from ECG recordings by measuring the R–R interval of each Q–R–S wave complex, expressed in beats min^{-1}. Each R–R interval was classified as occurring either during the breathing episode (eupneic) or during the pause between episodes (apneic). R–R intervals occurring during transition periods, i.e. within 5 s of the beginning or end of a breathing episode, were not analysed further. Mean apneic and eupneic heart rates were determined for each animal while breathing normocarbic and hypercarbic gas, before and after vagal blockade or a sham infusion of saline (normocarbia only). Mean apneic and eupneic heart rates of all animals were compared using a two-way two-factor repeated-measures analysis of variance (ANOVA) and Student–Newman–Keuls (SNK) multiple comparison test, $P<0.05$ (SigmaStat, Jandel Scientific).

Results

Initial investigations

During quiet euthermic wakefulness, animals maintained stable body temperatures of approximately 37 °C and exhibited mean heart rates in excess of 150 beats min^{-1} and continuous breathing with an average frequency of 35 breaths min^{-1} (Fig. 1A). Instantaneous heart rate was not constant during the respiratory cycle and was higher during inspiration (217±18 beats min^{-1}) than during the NVP (169±12 beats min^{-1}; mean ± S.E.M.). These differences amounted to a 28.8 % tachycardia associated with euthermic breathing (Fig. 2).

During hibernation, animals maintained their body temperatures approximately 1–2 °C higher than the ambient temperature. Heart rates decreased from euthermic levels to hibernation rates of less than 30 beats min^{-1}. Respiration showed a similar decrease from euthermic levels and, during deep hibernation, occurred in distinct 30–120 s episodes

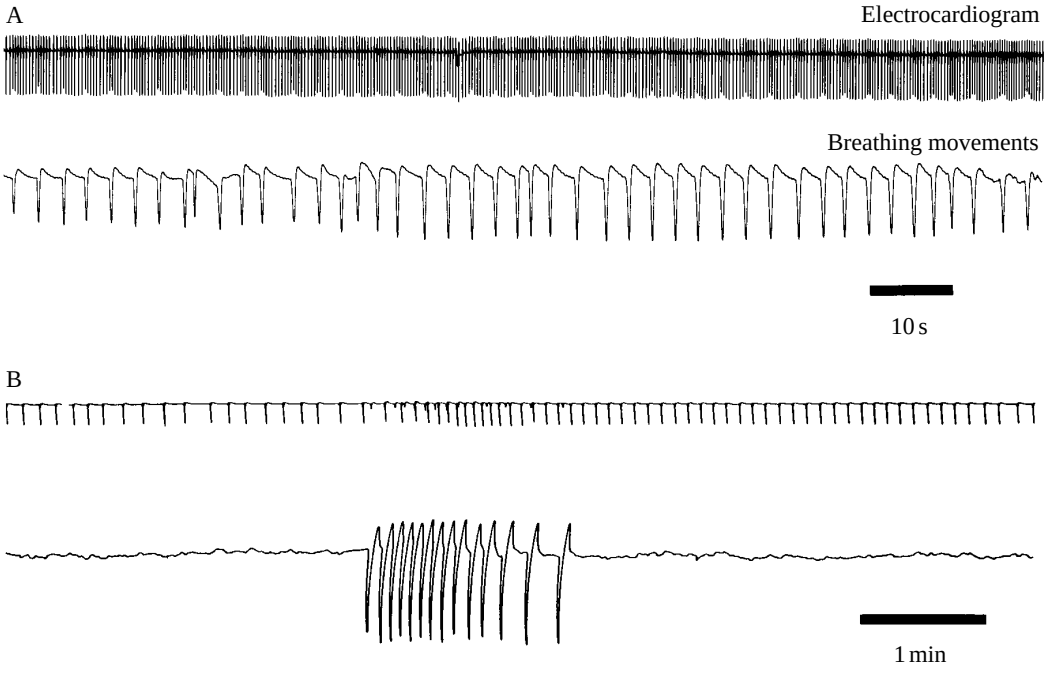

Fig. 1. A record of the electrocardiogram (upper trace) and breathing movements (lower trace) of a golden-mantled ground squirrel during (A) quiet euthermic wakefulness at room temperature and (B) hibernation at an ambient temperature of 5 °C. During euthermy, breathing is continuous and the ECG shows a tachycardia synchronous with the inspiratory phase (downward deflection) and bradycardia associated with the pause between breaths. During hibernation, breathing is episodic and the ECG shows a tachycardia synchronous with the eupneic period.

separated by apneic periods ranging between 5 and 30 min in duration (Fig. 1B).

When animals were breathing episodically, heart rate was significantly greater during eupnea than during apnea. Heart rates decreased with ambient temperature during hibernation, as did the absolute differences in heart rate during apnea and eupnea. Apneic *versus* eupneic heart rates (in beats min^{-1} ± S.E.M.) were 17.0±2.3 *versus* 35.3±7.8 at 15 °C, 12.1±0.9 *versus* 16.0±1.9 at 10 °C and 7.9±0.6 *versus* 8.67±0.3 at 5 °C, resulting in 108 %, 32 % and 11.5 % tachycardia, respectively (Fig. 2).

Vagal blockade study

Differences between apneic and eupneic heart rate were present in animals breathing both air and 3 % CO_2 at 5 °C (ANOVA, F=54.489, P=0.017; SNK at P<0.05, d.f.=9.0 during both treatments; Fig. 3). Animals exposed to hypercarbia had slightly higher mean heart rates (P<0.10), but did not show a more pronounced proportional tachycardia, than during normocarbia (Figs 3, 4). On average, animals exposed to air and 3 % CO_2 expressed an 11.5±2.6 % and 11.7±2.8 % increase in heart rate during breathing, respectively. Sham infusions were made to differentiate between the effects of vagal blockade and the infusion itself. A 0.04 ml infusion of saline had no effect on mean apneic or eupneic heart rate nor on the change in heart rate noted between apnea and eupnea (SNK at P<0.05, d.f.=8.6; Fig. 3). Infusions of 0.04 ml of 2 % lidocaine hydrochloride had no significant effect on eupneic heart rate during either normocarbic or hypercarbic exposure. Apneic heart rates, however, increased to corresponding eupneic levels (Fig. 3). The respiratory-related tachycardia was effectively abolished following vagal blockade. Mean apneic and eupneic heart rates were indistinguishable; the proportional tachycardia fell to approximately 2.2 % (Fig. 4).

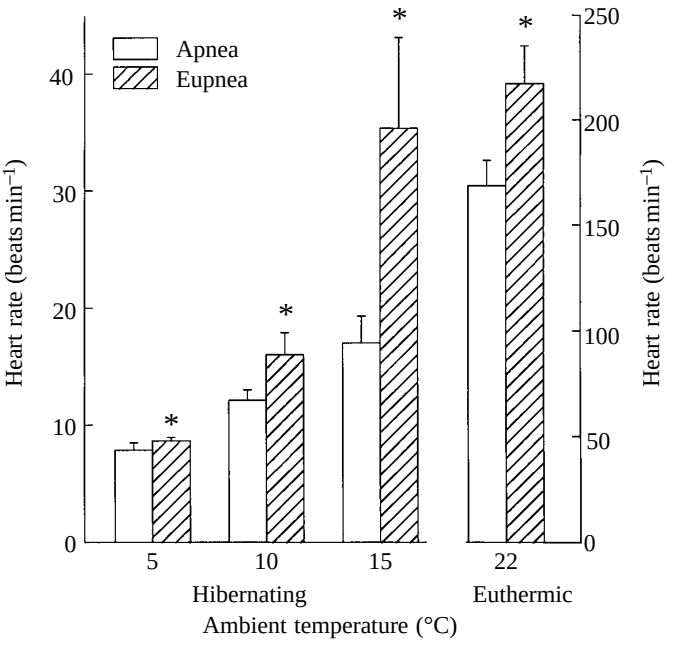

Fig. 2. Mean values with standard errors (N=5–6) for apneic (non-ventilatory period) and eupneic (inspiratory) heart rates of squirrels during hibernation at 15, 10 and 5 °C ambient temperatures (left-hand axis) and during quiet euthermic wakefulness at room temperature (right-hand axis). Note the change in scale of the vertical axis between hibernation and euthermia. All heart rates are significantly greater (*) during eupnea than during apnea at the same temperature (Student's t-test, P<0.05).

Discussion

During euthermia, golden-mantled ground squirrels breathe continuously (Fig. 1A) and exhibit cardiorespiratory coordination, resulting in a 28.8 % tachycardia during the inspiratory phase (Fig. 2). Similar coordination, referred to as respiratory-related sinus-arrhythmia, is present in many other species and is believed to be mediated by reductions of vagal tone resulting in phasic reductions of parasympathetic cardio-inhibition during inspiration (Anrep *et al.* 1936*a*,*b*; Angell James and De Burgh Daly, 1972; Johansen *et al.* 1977; Hirsch and Bishop, 1981; Eckberg, 1983; De Burgh Daly and Kirkman, 1989; Richter and Spyer, 1990; Novak *et al.* 1993).

Hibernating species typically display one of two distinctive ventilatory patterns. Ventilation either occurs as a continuous series of single breaths separated by short apneas (single-breath breathing) or as clusters of breaths separated by much longer apneas (episodic breathing) (Milsom, 1992). The golden-mantled ground squirrels used in the present study exhibited an episodic breathing pattern (Fig. 1B) while hibernating at 15, 10 and 5 °C ambient temperatures and had significantly higher heart rates during eupnea than during apnea (Fig. 2). These data indicate that cardiorespiratory coordination, similar to that occurring during euthermia, is maintained during hibernation. These observations do not, however, support or refute the existence of parasympathetic activity during hibernation. The ventilatory tachycardia could result either from the removal of parasympathetic cardio-inhibition or from sympathetic excitation during eupnea.

Mean heart rate was reduced in proportion to ambient temperature and to the body temperatures at which animals were hibernating (Fig. 2). It should be noted that animals were in a stable hibernation state at each of the three temperature, 15, 10 and 5 °C, as indicated by stable body temperatures within 2 °C of ambient and stable cardiac and respiratory rhythms over periods of several days. The ventilatory tachycardia was greatest at 15 °C and decreased at lower ambient temperatures. These data indicate that the reductions in ventilatory tachycardia between 15 and 5 °C were not the result of hibernation *per se*, but rather were determined by an additional effect of body temperature.

At 5 °C, alterations in heart rate between apnea and eupnea were small in absolute terms and reduced when compared with values at 15 and 10 °C. The increases in heart rate (10–12 %) during the breathing period were statistically significant, however. Assuming a constant stroke volume, this tachycardia

would have been manifested as a physiologically significant 10–12 % increase in cardiac output during the eupneic phase.

Animals exposed to 3 % CO_2 had slightly higher mean heart rates than when breathing air (Fig. 3). Mild tachycardia is commonly associated with hypercarbic exposure and has been previously documented in hibernating species (Lyman, 1951). McArthur and Milsom (1991) have shown that hibernating *S. lateralis*, when exposed to mild hypercarbia, increased their net breathing frequencies (from 2.59±0.30 to 5.89± 1.08 breaths min^{-1}). Although they had breathing episodes of similar duration (51.3±15.2 s *versus* 59.0±9.8 s) to those observed while they breathed air, they took a greater number of breaths per episode (8.8±1.0 *versus* 6.86±0.58). In the present study, heart rate in animals exposed to 3 % CO_2 was again elevated during the breathing episodes compared with that during apnea. Although this increase was larger in absolute terms than that in animals breathing air, it was exactly the same in relative (proportionate) terms. The degree of proportional tachycardia, therefore, appears to correspond to the duration of the breathing episodes, or to the initiation of the breathing episode, regardless of duration. Absolute changes in heart rate, however, may have been influenced by the number of breaths taken during these episodes.

Saline infusion into the vagal cuff had no effect on either mean heart rate or the change in heart rate noted during the transition from apnea to eupnea (Fig. 3). These observations indicate that no physiological changes resulted from fluid infusion into the cuff alone. Thus, the changes that did occur following Xylocaine infusion were the direct result of the pharmacological action of Xylocaine on the isolated nerve.

During exposure to both air and 3 % CO_2, Xylocaine infusion resulted in an increase in the mean apneic heart rate to levels equal to, or slightly greater than, the eupneic heart rate before blockade (Fig. 3). These results complement those of Milsom *et al.* (1993), who noted an 8–12 % increase in heart rate following bilateral vagotomy in anesthetized hibernating ground squirrels. These data confirm the presence of vagal parasympathetic tone during the apnea. They also demonstrate that the mechanisms responsible for elevating mean heart rate during hypercapnic exposure are unaffected by vagal blockade and, thus, are not mediated by the vagus.

Xylocaine infusion virtually abolished the difference in heart rate between apnea and eupnea (Fig. 4). The residual tachycardia (2.2 %) present following vagal blockade was comparable to that observed following total denervation of the heart in other species (Bernardi *et al.* 1989) and could be explained as the result of enhanced atrial filling by changes in intrathoracic negative pressure during breathing. These data illustrate that unobstructed vagal conduction is necessary for apneic bradycardia to occur and that phasic parasympathetic tone does influence heart rate during hibernation. Thus, these data suggest that the resting parasympathetic tone which dampens heart rate during apnea is totally removed during eupnea.

The alterations in heart rate between apnea and eupnea observed in hibernating *S. lateralis* were similar to the ventilatory tachycardia exhibited by many other episodically breathing animals (Angell James and De Burgh Daly, 1972). In addition, the proportional tachycardia noted during hibernation was similar to, or at times greater in magnitude than, that in the same species during euthermia. In general, ventilatory tachycardia has been attributed either to a simultaneous deactivation of cardiac vagal motor output associated with feedforward interactions (central 'irradiation') between respiratory neurons and cardiac vagal motorneurons within the medulla (Anrep *et al.* 1936*a*; Angell James and De Burgh Daly, 1972; Burggren, 1975; Hirsch and Bishop, 1981; Richter and Spyer, 1990) or to a feedback inhibition of cardiac vagal motor

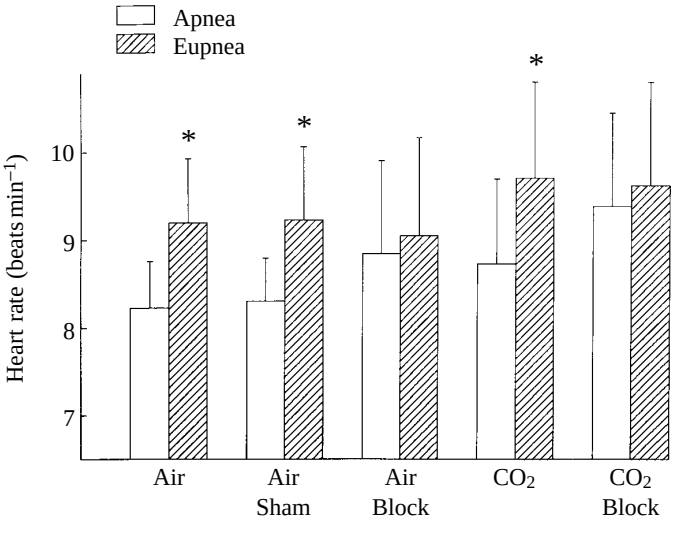

Fig. 3. Mean + S.E.M. (N=4) apneic and eupneic heart rate of all hibernating squirrels breathing air and 3 % inspired CO_2 before and after a sham infusion of saline (air only) and an infusion of 2 % Xylocaine (block). An asterisk denotes conditions where heart rate was significantly greater during eupnea than during apnea (ANOVA and SNK, P<0.05).

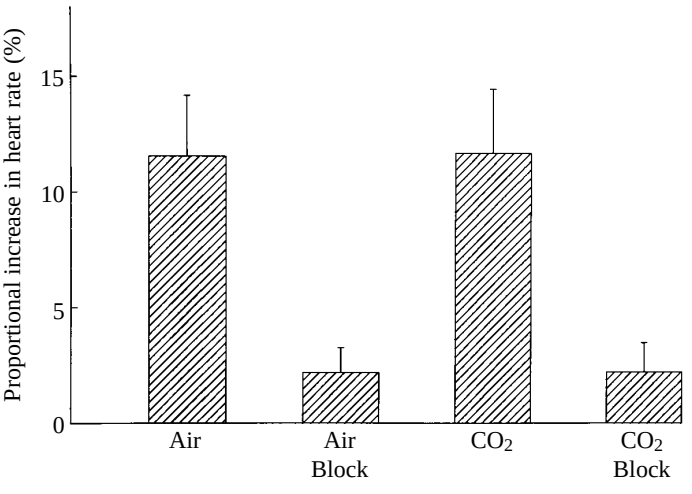

Fig. 4. Mean + S.E.M. (N=4) proportional increase in heart rate from apnea to eupnea of hibernating squirrels breathing air and 3 % inspired CO_2 before and after vagal blockade.

output, *via* a vagal pulmonary inflation reflex (Anrep *et al.* 1936*b*; Angell James and De Burgh Daly, 1972; Johansen *et al.* 1977; Hirsch and Bishop, 1981; Eckberg, 1983; De Burgh Daly and Kirkman, 1989; Richter and Spyer, 1990; Novak *et al.* 1993). Even though the former is only observed following periods of apnea, while the latter can occur on a breath-by-breath basis, distinguishing between these two mechanisms is difficult, particularly since both can act simultaneously (Anrep *et al.* 1936*a*). Importantly, however, both mechanisms remove vagal inhibition to the heart during breathing; thus, both require that a level of resting parasympathetic tone be present during the apnea. Vagal blockade in the present study would remove this tone as well as any vagal sensory input which might have been involved in feedback inhibition.

The vagus nerve is also known to carry sympathetic postganglionic neurons, and many mammalian hearts do receive substantial sympathetic input *via* this route. The observed ventilatory tachycardia could not have been the result of sympathetic stimulation of heart rate during the breathing episode, however. If such vagus-mediated sympathetic activation were responsible for ventilatory tachycardia, heart rate would have decreased to apneic levels following vagal blockade. The observed elevation of heart rate to eupneic levels, therefore, supports the hypothesis that parasympathetic cardiac inhibition, rather than sympathetic stimulation, was removed by vagal blockade. This is consistent with reports that hibernating mammals have sparse sympathetic innervation of this type (Nielsen and Owman, 1968; Nilsson, 1983).

In an earlier study, Lyman (1982) did not observe respiratory-related changes in heart rate in *S. lateralis* during hibernation and concluded that ventilatory and heart rates were far too low for such changes to occur. One possible reason for this discrepancy between studies may arise from differences in the patterns of breathing employed by the animals during the different investigations. As mentioned earlier, some species breathe with a series of evenly spaced individual breaths while some display distinct episodes of continuous breathing. These latter species, when disturbed, have also been shown to switch to a less episodic pattern. When heart rates are high, the duration of a single breath will span a number of individual heart beats. A relatively small transient decrease in cardiac vagal tone will translate into a noticeable increase in instantaneous heart rate for a number of heart beats. During hibernation, however, mean heart rate drops from approximately 250 beats min^{-1} to less than 10 beats min^{-1} (Burlington and Darvish, 1988). Now, a single breath will influence fewer heart beats; in fact, it may occur between beats and have no effect at all. The decrease in vagal tone resulting from a series of consecutive breaths in an episode, however, may have a more pronounced effect. During episodic breathing, decreases in cardiac vagal tone may occur over a longer period, thereby influencing a series of consecutive heart beats. Ventilatory tachycardia during hibernation may, therefore, only be discernible when breaths are clustered into distinct episodes.

The physiological significance of ventilation tachycardia (and apneic bradycardia) during episodic breathing has been reviewed extensively (Angell James and De Burgh Daly, 1972). It is believed that the primary benefits derive from increasing tissue and pulmonary flows at a time when gas exchange between tissue stores and atmospheric air can be maximized, i.e. during ventilation. A recent model proposed by Rapoport *et al.* (1993) supports this conclusion. The model demonstrates that, during episodic breathing, low apneic heart rates and ventilatory tachycardia produce lower venous levels of CO_2 and reduced arterial CO_2 fluctuations compared with those during a constant cardiac output. Although an 11.5 % increase in heart rate does not seem much, by potentially shortening the length of the ventilatory episode required to re-establish blood gas homeostasis, it could dramatically reduce energy expenditure. Similarly, parasympathetic inhibition of heart rate during apnea will decrease the work done by the heart and the metabolic costs of tissue and pulmonary perfusion during this phase, when ventilation is not occurring.

The predominant view has been that the parasympathetic nervous system was relatively unimportant to autonomic control and greatly overshadowed by the sympathetic system during deep hibernation. Evidence for this general conclusion was primarily derived from the supposed absence of parasympathetic cardiovascular regulation during hibernation (see Lyman, 1982, for a review). The present study, however, demonstrates that there is resting parasympathetic tone in the cardiac vagus during hibernation and that this resting tone fluctuates in a regular manner in association with respiration. The data also indicate that the parasympathetic influence on heart rate is more dependent on body temperature than on hibernation *per se* and, thus, it will be more predominant in animals hibernating at higher body temperatures. Although the corresponding changes in the influence of body temperature on sympathetic function and on the relative influence of sympathetic *versus* parasympathetic tone during hibernation have not yet been assessed, these results support the hypothesis that parasympathetic motor drive is present during deep hibernation.

Funding for this study was provided by the Natural Sciences and Engineering Research Council of Canada, through a general operating grant (W.K.M.) and postgraduate scholarship (M.B.H.). We are grateful to Rohan Bissoondath, Michelle Calder and Sarah Franks for assistance with some of the data collection. The authors also wish to acknowledge the assistance, comments and critical insights provided by participants of 'Living in the Cold III' the International Hibernation Symposium at Mount Crested Butte, Colorado, where portions of this study were presented as a poster. Preliminary accounts of this study have been published in abstract form (Harris and Milsom, 1994*b*).

References

ANGELL JAMES, J. AND DE BURGH DALY, M. (1972). Some mechanisms involved in the cardiovascular adaptations to diving. In *The Effects of Pressure on Organisms. Symp. Soc. exp. Biol.* **XXVI**, 313–341. Cambridge: Cambridge University Press.

ANREP, G. V., PASCUAL, W. AND ROSSLER, R. (1936*a*). Respiratory variations of the heart rate. II. The central mechanism of the respiratory arrhythmia and the inter-relations between the central and reflex mechanisms. *Proc. R. Soc. Lond. B* **119**, 218–230.

ANREP, G. V., PASCUAL, W. AND ROSSLER, R. (1936*b*). Respiratory variations of the heart rate. I. The reflex mechanism of the respiratory arrhythmia. *Proc. R. Soc. Lond. B* **119**, 191–217.

BERNARDI, L., KELLER, F., SANDERS, M., REDDY, P. S., GRIFFITH, B., MENO, F. AND PINSKY, M. R. (1989). Respiratory sinus arrhythmia in the denervated heart. *J. appl. Physiol.* **67**, 1447–1455.

BURGGREN, W. W. (1975). A quantitative analysis of ventilatory tachycardia and its control in two chelonians, *Pseudemys scripta* and *Testudo graeca*. *J. exp. Biol.* **63**, 367–380.

BURLINGTON, R. F. AND DARVISH, A. (1988). Low-temperature performance of isolated working hearts from a hibernator and a non hibernator. *Physiol. Zool.* **61**, 387–395.

BURLINGTON, R. F. AND MILSOM, W. K. (1989). The cardiovascular system in hibernating mammals: recent advances. In *Living in the Cold II* (ed. A. Mallan and B. Canguilhem), pp. 235–243. Colloque INSERM/John Libbey Eurotext Ltd.

DE BURGH DALY, M. AND KIRKMAN, E. (1989). Differential modulation by pulmonary stretch afferents of some reflex cardioinhibitory responses in the cat. *J. Physiol., Lond.* **417**, 323–341.

ECKBERG, D. L. (1983). Human sinus arrhythmia as an index of vagal cardiac outflow. *J. appl. Physiol.* **54**, 961–966.

HARRIS, M. B. AND MILSOM, W. K. (1994*a*). The ventilatory response to hypercapnia in hibernating golden-mantled ground squirrels. *Physiol. Zool.* **67**, 739–755.

HARRIS, M. B. AND MILSOM, W. K. (1994*b*). The role of the vagus nerve in cardio-respiratory control during hibernation. *FASEB J.* (Abstracts) **8**, A1.

HIRSCH, J. A. AND BISHOP, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *Am. J. Physiol.* **241**, H620–H629.

JOHANSEN, K., BURGGREN, W. W. AND GLASS, M. (1977). Pulmonary stretch receptors regulate heart rate and pulmonary blood flow in the turtle, *Pseudemys scripta*. *Comp. Biochem. Physiol.* **58**A, 185–191.

LYMAN, C. P. (1951). Effect of increased CO_2 on respiration and heart rate of hibernating hamsters and ground squirrels. *Am. J. Physiol.* **167**, 638–643.

LYMAN, C. P. (1982). The hibernating state. In *Hibernation and Torpor in Mammals and Birds* (ed. C. P. Lyman, J. S. Willis, A. Malan and L. C. H. Wang), pp. 54–76. New York, London: Academic Press.

LYMAN, C. P. AND O'BRIEN, R. C. (1963). Autonomic control of circulation during the hibernating cycle in ground squirrels. *J. Physiol., Lond.* **168**, 477–499.

MCARTHUR, M. D. AND MILSOM, W. K. (1991). Changes in ventilation and respiratory sensitivity associated with hibernation in Columbian (*Spermophilus columbianus*) and golden-mantled (*Spermophilus lateralis*) ground squirrels. *Physiol. Zool.* **64**, 940–959.

MILSOM, W. K. (1992). Control of breathing in hibernating mammals. In *Physiological Adaptations in Vertebrates: Respiration, Circulation and Metabolism* (ed. S. C. Wood, R. E. Weber, A. R. Hargens and R. W. Millard), pp. 119–147. New York: Marcel Dekker.

MILSOM, W. K., BURLINGTON, R. F. AND BURLESON, M. L. (1993). Vagal influence on heart rate in hibernating ground squirrels. *J. exp. Biol.* **185**, 25–32.

NEILSEN, K. C. AND OWMAN, C. H. (1968). Differences in cardiac adrenergic innervation between hibernators and non-hibernating mammals. *Acta. physiol. scand.* **74** (Suppl. **316)**, 1–30.

NILSSON, S. (1983). The mammalian heart. In *Autonomic Nerve Function in Vertebrates*, pp. 137–138. New York: Springer-Verlag.

NOVAK, V., NOVAK, P., DECHAMPLAIN, J., LEBLANC, A. R., MARTIN, R. AND NADEAU, R. (1993). Influence of respiration on heart rate and blood pressure fluctuations. *J. appl. Physiol.* **74**, 617–626.

O'SHEA, J. E. (1987). Temperature sensitivity of cardiac muscarinic receptors of bat atria and ventricle. *Comp. Biochem. Physiol.* **86**C, 365–370.

O'SHEA, J. E. AND EVANS, B. K. (1985). Innervation of bat heart: cholinergic and adrenergic nerves innervate all chambers. *Am. J. Physiol.* **249**, H876–H882.

RAPOPORT, D. M., NORMAN, R. G. AND GOLDRING, R. M. (1993). CO_2 homeostasis during periodic breathing: predictions from a computer model. *J. appl. Physiol.* **75**, 2302–2309.

RICHTER, D. W. AND SPYER, K. M. (1990). Cardiorespiratory control. In *Central Regulation of Autonomic Function* (ed. A. D. Loewy and K. M. Spyer), pp. 189–207. Oxford: Oxford University Press.

TWENTE, J. W. AND TWENTE, J. (1978). Autonomic regulation of hibernation by *Citellus* and *Eptesicus*. In *Strategies in the Cold: Natural Torpor and Thermogenesis* (ed. L. Wang and J. W. Hudson), pp. 327–373. New York: Academic Press.