J. exp. Biol. ***185****, 25–32 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

VAGAL INFLUENCE ON HEART RATE IN HIBERNATING GROUND SQUIRRELS

WILLIAM K. MILSOM

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

ROY F. BURLINGTON

Department of Biology, Central Michigan University, Mount Pleasant, MI 48859, USA

AND MARK L. BURLESON

Department of Biology, University of Texas, Arlington, TX 76019, USA

Accepted 7 July 1993

Summary

The heart rate of anesthetized golden mantled ground squirrels (*Spermophilus lateralis*) falls from 372±20 to 37±9beatsmin^{-1} during hibernation at 7°C body temperature. Heart rate in the hibernating animals often waxed and waned in a fashion that was not clearly linked to the breathing pattern. Similar observations have been made on unanesthetized ground squirrels. Under anesthesia, the effects of vagotomy were small in both euthermic and hibernating animals and led to a 6–8% increase in heart rate. Vagotomy also eliminated the cyclic fluctuations of heart rate in hibernating animals exhibiting this phenomenon. The post-vagotomy heart rate exhibited by these individuals suggested that both sympathetic excitation and parasympathetic depression were involved in producing these cyclic changes. Vagal stimulation reduced mean heart rate by at most 60–80% in euthermic and hibernating animals. The strength of the stimulus required to elicit a maximal response in the hibernating animals was 35–45% greater than that required in euthermic animals. Comparisons of mean heart rates obtained from euthermic and hibernating animals which were vagotomized, intact or stimulated to produce a maximum bradycardia produced temperature quotients of 2.21, 2.15 and 2.31, respectively. In this species, both resting vagal tone and the effects of vagal stimulation decrease in parallel with decreasing temperature over the range studied.

Introduction

In the preceding paper, it was shown that the coronary circulation of ground squirrels (*Spermophilus lateralis*), unlike that of most other mammals, was relatively insensitive to infusion of acetylcholine. This was particularly true at the lower temperatures characteristic of those experienced by these animals during hibernation. This suggested that the coronary circulation of these animals was under very little parasympathetic

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

control (Burlington and Milsom, 1993). It has previously been argued that the influence of the parasympathetic nervous system on the heart rate of hibernating species also declines as they enter hibernation (Lyman, 1982). Once deep hibernation has been reached, its effect is minimal, if not completely absent. This also appears to be the case during the period of arousal from hibernation (Lyman and O'Brien, 1964; Johansen *et al.* 1964; Lyman, 1982).

The mechanism(s) which underlie this reduction and/or elimination of parasympathetic control of heart rate in deep hibernation is unknown. It could be due to reduced impulse propagation along the vagus or to a reduction or cessation of acetylcholine production at the nerve endings (Johansen *et al.* 1964). If the heart rate of animals in deep hibernation is accelerated, infusion of acetylcholine can cause a bradycardia but the dose required increases with the time the animal has been in deep hibernation. This suggested that the sensitivity of the heart to acetylcholine also decreased (Lyman and O'Brien, 1964; Biewald and Raths, 1959). The absence of parasympathetic control during arousal appears to be due to the maximal stimulation of the sympathetic nervous system and central withdrawal of parasympathetic tone. Vagal stimulation or acetylcholine infusion during this period will reduce heart rate (Lyman and O'Brien, 1964; Johansen *et al.* 1964; Lyman, 1982).

The above data suggest that the reduction in parasympathetic control of heart rate during hibernation may stem from changes in any, or all, of central motor output, vagal conduction, vagal transmitter release or cardiac sensitivity to acetylcholine. Given this range of possibilities, the present study was designed to determine simply whether vagal activation can alter heart rate during hibernation at 7°C.

Materials and methods

Adult golden mantled ground squirrels [*Spermophilus lateralis* (Say)] were purchased from a collector in Redding, California, USA. From June to October the animals were housed in a controlled-environment chamber at an ambient temperature (T_a) of 20±1°C under a 12h:12h L:D photoperiod (lights on 06:00h) and were fed laboratory chow supplemented with carrots, apples and sunflower seeds *ad libitum*. The squirrels were induced to hibernate in late November by gradually reducing T_a and the light phase of the photoperiod. Over 2 weeks, T_a was decreased to 5±1°C and the photoperiod was changed to 2h:22h L:D (lights on 10:00h). During this period, the ground squirrels had access to laboratory chow and water *ad libitum* but were not handled or disturbed. Most animals had begun to hibernate before the end of the induction period and all were hibernating within 2 weeks of exposure to the final regime. These environmental conditions were maintained throughout the winter. Experiments were performed between January and March. All procedures were in keeping with the guidelines set out by the Canadian Council on Animal Care.

Six animals were studied during periods of periodic arousal when their body temperatures (T_b) were approximately 37°C; nine animals were studied while in deep hibernation (T_b approximately 7°C). 1h prior to an experiment a squirrel was injected with sodium pentobarbital (Somnotol; 30–65mgkg^{-1}) intraperitoneally. The animal was

removed from the environment chamber and brought into the laboratory. The hibernating animals were packed on ice to maintain a depressed T_b. A needle thermometer (BAT 12, Sensortex) was inserted into the body cavity and T_b was monitored continuously throughout the experiment. Experiments were short, and it was not difficult to maintain animals at a constant body temperature. A midline incision was made in the ventral neck from jaw to sternum after infiltrating the area with local anesthetic (Lidocaine 2%). The vagi were exposed bilaterally and carefully cleared of surrounding tissue. Platinum bipolar stimulating electrodes were placed under the vagus on one side (chosen randomly). An electrocardiogram (ECG) was obtained from needle electrodes placed across the chest with a ground electrode inserted into one hindlimb. The signal was amplified (Gould, universal amplifier) and displayed on a polygraph recorder. Heart rate was monitored for 10–15min and then the vagus nerves were sectioned, one at a time, while continuously monitoring the heart rate. Following this, a series of stimulations (6ms pulses at 100Hz) was applied to the posterior end of the cut vagus *via* the bipolar electrodes (Grass model S6). The voltage was slowly increased until a threshold was reached which just caused heart rate to slow. The voltage was then increased in steps until further increases ceased to cause any further decrease in heart rate. The threshold voltage was then reconfirmed and the procedure was repeated on the contralateral vagus nerve.

Results

Effects of vagotomy

In many of the hibernating animals, resting heart rate showed periods of acceleration and deceleration (Fig. 1). This is not an artifact of the experimental situation as it has been observed in undisturbed animals in deep hibernation by many researchers (Strumwasser, 1959; Lyman and O'Brien, 1964). Vagotomy invariably eliminated this pattern and produced a regular heart rate, which was always intermediate in value between the fastest and slowest rates observed in the oscillating pattern (Fig. 2). The net effect of vagotomy was generally small. In euthermic (T_b=37˚C) animals (Table 1), it increased heart rate by only 8%. In the hibernating animals, it increased heart rate by 6 % on average. In this latter group, however, five animals showed no change in mean heart rate on vagal section and four animals exhibited an increase in heart rate of approximately 12%. Two things are of note here. The first is that, although mean heart rate did not always change on vagotomy in hibernating animals, this did not mean that vagotomy was without effect. This is clearly depicted in Fig. 2 where vagotomy eliminated the oscillating pattern of acceleration and deceleration of heart rate without affecting the mean rate. The second is that vagotomy did not always cause an increase in heart rate in euthermic animals either: heart rate remained unchanged in three of the six animals.

Effects of vagal stimulation

In all euthermic animals, vagal stimulation decreased heart rate (Fig. 3, Table 1). When using trains of 6ms pulses at 100Hz, 4.1V was required on average to elicit any response and 6.3V was required to elicit a maximal response. The maximal response was, on average, a 64% reduction in heart rate. In hibernating animals, the five animals that

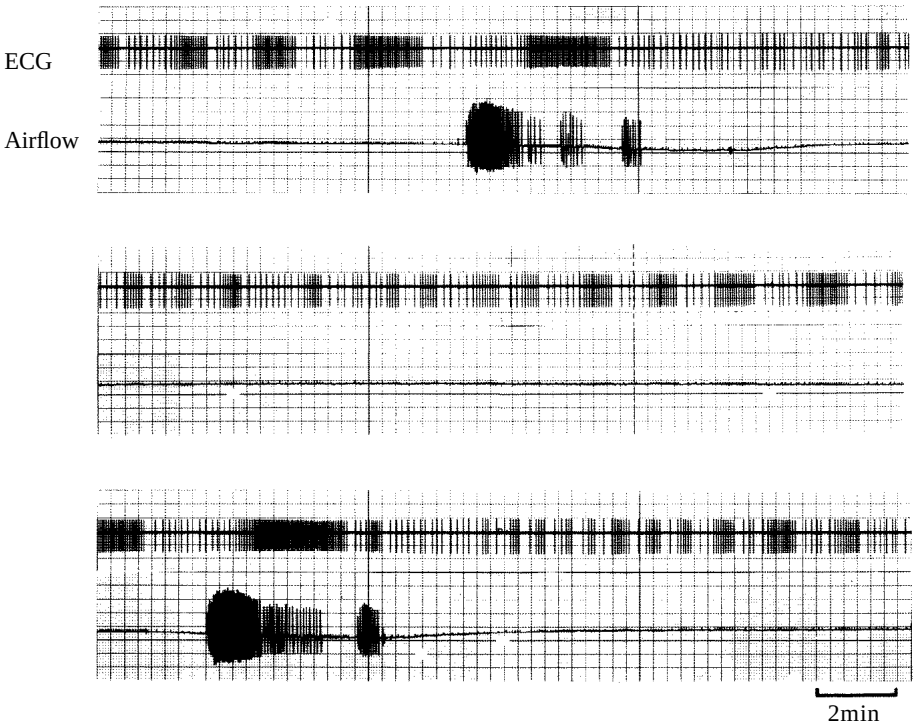

Fig. 1. ECG and airflow traces depicting heart rate and breathing of a golden mantled ground squirrel in deep hibernation. Traces are continuous. Despite an apnea of almost 30min between breathing episodes (indicative of deep, undisturbed hibernation), the heart shows episodes of acceleration and deceleration.

Table 1. *Effect of body temperature on vagal function in golden mantled ground squirrels*

	Euthermic	Hibernating		
Body temperature (°C)	37.5±0.2 (6)	7.2±0.2 (9, all animals)	7.3±0.3 (4[a])	7.2±0.2 (5[a])
Heart rate (beatsmin^{-1})				
Vagotomized	402±8	41±7	57±12	16±2
Intact	372±20	37±9	51±13	16±2
Vagal stimulation	135±19	11±1	8±1	16±2
Voltage (V)				
Threshold	4.1±0.4		5.9±0.7	
Maximum response	6.3±0.6		8.6±1.1	

[a]The nine hibernating animals fell into two groups (see text), which are presented separately. Numbers of animals are given in parentheses.

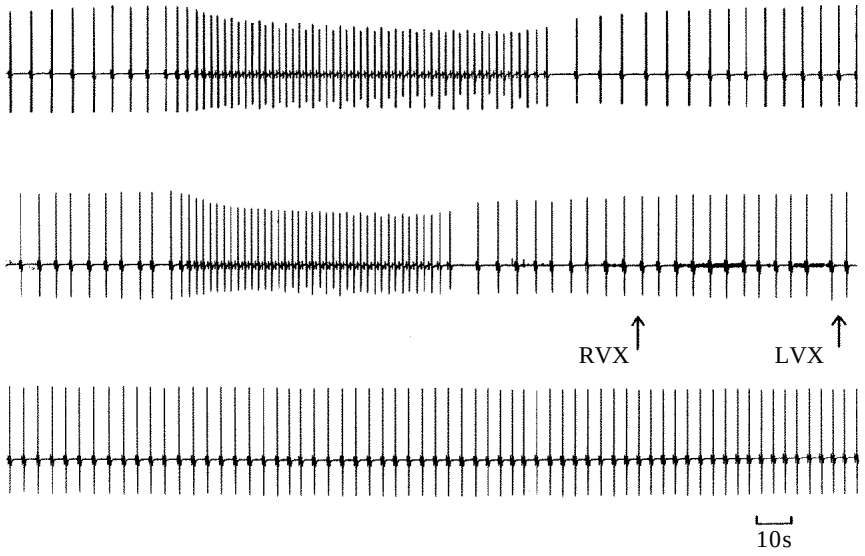

Fig. 2. Effect of bilateral vagotomy on the heart rate of an anesthetized, hibernating squirrel exhibiting an oscillating pattern of accelerating and decelerating heart rate. RVX, right vagus cut; LVX, left vagus cut. The traces are continuous.

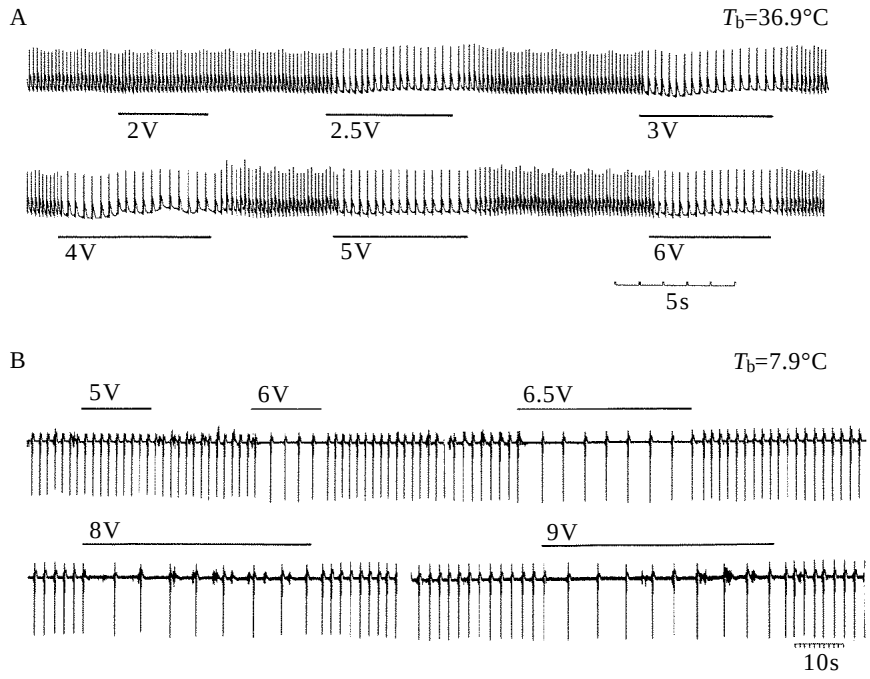

Fig. 3. Effect of vagal stimulation on the heart rate of anesthetized, euthermic (A) and hibernating (B) squirrels. The nerve was stimulated with 6ms pulses at 100Hz for the durations (bars) and voltages indicated. Note the difference in time scales between the two sets of traces.

did not show a change of mean heart rate following vagal section did not respond to vagal stimulation (Table 1), whereas the four animals whose mean heart rate did change following vagal section also responded to vagal stimulation. On average, 5.9V was required to elicit a response and 8.6V was required to elicit the maximal response of an 84% reduction in heart rate in the latter group (Table 1). If all hibernating animals are treated as one group, this amounts to a mean reduction of 71% in heart rate for the group.

Discussion

The results of the present investigation suggest that there is very little vagal parasympathetic tone acting on heart rate in euthermic squirrels. In hibernating squirrels, this influence is reduced or absent. This is consistent with previous studies (Biewald and Raths, 1959; Lyman and O'Brien, 1964; Johansen *et al.* 1964) as well as with observations that infusion of atropine often did not increase heart rate in hibernating animals (Lyman and O'Brien, 1964). Nonetheless, vagal stimulation can reduce heart rate by, at most, two-thirds in euthermic animals. In hibernating squirrels, in contrast, vagal stimulation only reduced heart rate in half of the animals. This could not be accounted for by differences in body temperature; nor could it really be accounted for by differences in resting heart rate. Although the mean heart rate of the hibernating squirrels that did respond to vagal stimulation was higher than that of the group that did not, one individual in the former group had a resting heart rate of 16beatsmin^{-1} (the same as the mean heart rate of the group exhibiting no response) which was reduced to 6beatsmin^{-1} on vagal stimulation. Previous investigators have been unable to elicit an effect of vagal stimulation in animals in deep hibernation (Biewald and Raths, 1959; Lyman and O'Brien, 1964; Johansen *et al.* 1964). They also concluded that this was not an effect of body temperature. Lyman and O'Brien (1964) concluded that it was a consequence of resting heart rate because, in their study, vagal stimulation was only effective if heart rate was elevated. The reasons for the difference between previous studies and ours in the ability of vagal stimulation to elicit bradycardia during deep hibernation are not clear, but may include our choice of stimulus parameters and the use of anesthesia.

These data clearly show that, although the stimulation voltages required to elicit a response were elevated, the vagus could conduct impulses at 7°C and that acetylcholine must have been released. This suggests that the absence of a vagal response in some animals was not due to changes in vagal function. Although differences in the sensitivity of cardiac cells to acetylcholine could be involved (Biewald and Raths, 1959; Lyman and O'Brien, 1964), there is a more plausible explanation. A possible clue comes from the animals that showed the oscillating pattern of accelerating and decelerating heart rate (Figs 1 and 2). In the animal depicted in Fig. 2, mean heart rate did not change upon vagotomy although the accelerations and decelerations in heart rate disappeared, suggesting that they were vagally mediated. The observation that heart rate did not change to the peak heart rate seen in the oscillating series suggests that vagotomy removes both excitatory as well as inhibitory inputs. This is supported by the observation that atropine infusion under these conditions also stabilizes heart rate at an intermediate level (Lyman, 1982). The further observation that vagal stimulation now failed to lower

heart rate to the lowest levels seen in the oscillating series suggests that vagal stimulation co-activates both the efferent excitatory and inhibitory pathways. It is well known that the vagus nerve contains both parasympathetic and sympathetic cardiac fibers. Typically, the parasympathetic response to vagal stimulation predominates and causes bradycardia. Differences in the extent to which vagal section and stimulation affected sympathetic or parasympathetic fibers in different individuals may explain the differences in the effects of these procedures on heart rate. If this is the case, then the data obtained in the subgroup of hibernating animals in which vagotomy increased heart rate may more clearly represent the role of vagal parasympathetic fibers in hibernating animals. On the basis of our data, however, we cannot distinguish between this possibility and the possibility that there was a decrease in the sensitivity of cardiac cells in some individuals.

There is also one further possibility. It is well documented that hibernating rodents cycle through repetitive bouts of hibernation interspersed by very short periods of periodic arousal. Once an animal has entered a bout of hibernation, it becomes progressively more sensitive to external stimuli, or more irritable, until it undergoes the next periodic arousal (Twente and Twente, 1967). We did not keep track of when in an individual bout of hibernation each animal was selected for experimentation. It is possible, therefore, that the animals that responded to vagal stimulation were selected at a later time in their individual bouts of hibernation than the animals that did not respond.

Keeping in mind the large variability in the effects of vagotomy and vagal stimulation on heart rate in both euthermic and hibernating individuals, the mean data for all animals

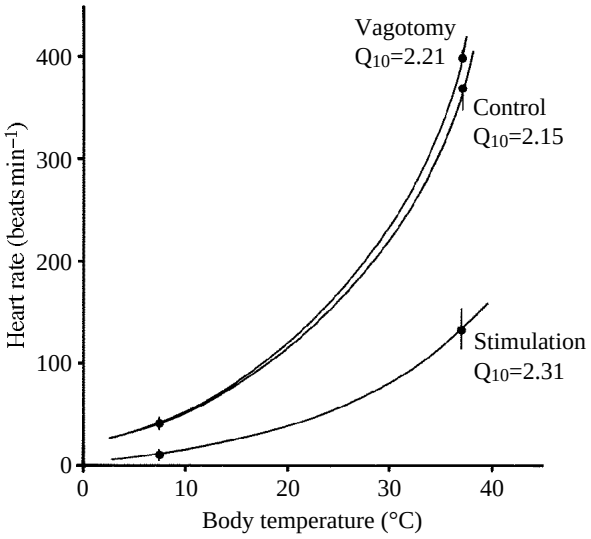

Fig. 4. Effect of body temperature on heart rate in golden mantled ground squirrels. Curves represent data from intact animals (control), vagotomized animals (vagotomy) and vagally stimulated animals (maximum response; stimulation). The numbers represent the Q_{10} values determined from the raw data. Curves were drawn on the basis of the Q_{10} values and the data at 37˚C. Values are means ±1 S.E.M., *N*=6 for euthermic (37˚C) animals and 9 for hibernating (7˚C) animals.

are presented in Fig. 4 to show the net effects of changing temperature on vagal control of heart rate. The curves in this figure were drawn on the basis of the Q_{10} values for data pairs and the 37˚C data point. This illustration reinforces the idea that there is very little resting vagal tone in these animals. It also suggests, however, that the vagal influence on heart rate (i.e. the extent to which vagal input can influence heart rate) is only reduced proportionately with body temperature and that the potential for parasympathetic control remains. This does not challenge current concepts about the dominant role of the sympathetic nervous system in the control of cardiovascular function during hibernation and arousal, nor does it challenge the idea that there is a reduction in parasympathetic control. It does, however, support early ideas suggesting that parasympathetic control does play a role in cardiovascular control in hibernating mammals (Britton, 1928; Strumwasser, 1960) and suggests that the decline in this control during entrance into hibernation is not due to withdrawal of descending input to vagal motor neuron pools, changes in vagal conduction or desensitization of cardiac cells, but is simply due to a proportionate reduction in vagal influence.

This research was funded by a Research Professor Award from Central Michigan University and by the NSERC of Canada. We are grateful to Cheryl Webb for the traces displayed in Fig. 1.

References

BIEWALD, G.-A. AND RATHS, P. (1959). Die chronotrope Vaguswirkung auf das Hamsterherz unter dem Einfluss von Winterschlaf und Hypothermie. *Pflügers Arch.* **268**, 530–544.

BRITTON, S. W.(1928). Effect of partial sympathectomy and vagotomy upon the course of hibernation in heterothermous animals. *C. r. Acad. Sci. U.S.S.R.* **73**, 1305–1308.

BURLINGTON, R. F. AND MILSOM, W. K.(1993). Differential effects of acetylcholine on coronary flow in isolated hypothermic hearts from rats and ground squirrels. *J. exp. Biol.* **185**, 17–24.

JOHANSEN, K., KROG, J. AND REITE, O. (1964). Autonomic nervous influence on the heart of the hypothermic hibernator. *Annls Acad. Sci. Fenn. A4* **71**, 243–255.

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: Academic Press.

LYMAN, C. P. AND O'BRIEN, R. C. (1964). Autonomic control of circulation during the hibernating cycle in ground squirrels. *J. Physiol., Lond.* **168**, 477–499.

STRUMWASSER, F. (1959). Regulatory mechanisms, brain activity and behavior during deep hibernation in the squirrel, *Citellus beecheyi. Am. J. Physiol.* **196**, 23–30.

STRUMWASSER, F. (1960). Some physiological principles governing hibernation in *Citellus beecheyi*. *Bull. Mus. comp. Zool.* **124**, 285–320.

TWENTE, J. W. AND TWENTE, J. A.(1967). Progressive irritability of hibernating *Citellus lateralis. Comp. Biochem. Physiol.* **25**, 467–474.