

EFFECT OF TEMPERATURE ON CARDIAC VAGAL ACTION IN THE TOAD *BUFO MARINUS*

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Summary

The effect of temperature on the action of the vagus nerve on the heart was studied in the toad *Bufo marinus*. Experiments were performed on two groups of toads, in one the heart was perfused at a constant rate with oxygenated Ringer's solution and in the other the circulation was left intact. In all toads there was a linear relationship between pulse interval (PI) and the frequency of vagal stimulation (f_v) at any one temperature. The slope of this relationship changed with temperature, the effectiveness of the vagus ($\Delta\text{PI}/\Delta f_v$) increasing with decreasing temperature. At low temperatures the vagus nerves of intact toads were more effective than in those with perfused hearts. It is suggested that, in intact toads at low temperatures, cardiac output decreases and the consequent accumulation of acetylcholine leads to increased vagal effectiveness.

Introduction

It is well known that heart rate increases with increasing temperature in ectothermic animals. This is in part due to a direct effect of temperature on pacemaker cells (Clark, 1920), but may also be influenced by the effect of temperature on important cardiovascular nerves and reflexes. This paper considers how one of those cardiovascular nerves, the vagus nerve, may have different effects on the heart at different temperatures in a toad and may thus affect the heart rate. The vagus nerve plays an important role in regulation of heart rate in amphibians, especially in association with diving and lung inflation (Jones, 1966) and in the control of blood pressure (Van Vliet and West, 1987).

There are two ways in which the vagus nerve can produce its effects on the heart: one is by a change in the frequency of action potentials arriving at the heart and the other is by peripheral effects at the neuroeffector junction, causing each action potential arriving at the heart to have a greater or lesser effect. Influences of this latter kind, whereby efferent nerve impulses travelling in the vagus are potentiated or inhibited according to local conditions at the heart, have been shown to operate in several circumstances in mammals, including during temperature changes. For example, vagal effectiveness is potentiated by hypoxia (Courtice

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et al. 1983) and by acidosis (Potter *et al.* 1986) and is reduced by the octapeptide angiotensin II (Potter, 1982) and increasing temperature (Potter *et al.* 1985). Potentiation of vagal action during hypoxia has been demonstrated in lizards also (Courtice, 1985).

Potter *et al.* (1985) showed that, in dogs, the effectiveness of the vagus increases dramatically as temperature decreases over a narrow range of temperatures (35–41 °C). If such a phenomenon occurs also in ectothermic animals, it may have far-reaching physiological consequences in these animals, whose body temperature may vary widely on a daily basis. Thus, it was decided to investigate whether this change in effectiveness of the cardiac vagus nerves occurs over a wide range of temperatures in the Queensland cane toad *Bufo marinus*.

Materials and methods

Ten toads of either sex weighing 100–150 g were stunned by a blow to the head and pithed just prior to the experiments. A midline, ventral incision was made and the pectoral girdle and xiphoid process were removed to expose the heart and major vessels.

Because cardiac output changes with body temperature in the toad, and changes in flow through the heart can alter the effectiveness of the vagus (Courtice and McCloskey, 1985) five toads were studied under conditions of controlled perfusion of the sinus venosus and right atrium by methods described previously (Courtice and McCloskey, 1985). A large (i.d. 2.0 mm), non-occlusive, nylon catheter was tied into the right atrium with a purse-string suture and left open to the atmosphere to drain the right atrium effectively. Another nylon catheter (i.d. 1.2 mm) was introduced into the anterior abdominal vein pointing towards the heart. Through this catheter, oxygenated amphibian Ringer's solution (110 mmol l⁻¹ NaCl, 1 mmol l⁻¹ KCl, 2.4 mmol l⁻¹ NaHCO₃, 5.5 mmol l⁻¹ glucose, 1.4 mmol l⁻¹ CaCl₂) was introduced into the sinus venosus and right atrium from a reservoir. The solution escaped through the atrial 'drain'. The flow was measured in a drip chamber inserted between the reservoir and the anterior abdominal vein. The height of the reservoir remained constant and the flow, as measured, varied by less than 20% in any one animal. The temperature of the perfusate was varied between 10 and 37 °C. Each of five animals was tested at four different temperatures within this range. Measurements of beat-by-beat pulse interval were recorded continuously on a Grass polygraph, *via* a Grass tachograph. The tachograph was triggered from a tension record from the beating heart. To obtain the tension record of cardiac contraction, a thread was tied through the apex of the ventricle and attached to a force transducer. In two animals which were prone to atrioventricular block, a ligature was tied between the atria and the ventricle and the ventricle was removed. A thread was then tied through the atrial wall and used to measure atrial contractions. In toads, the vagus nerves carry sympathetic fibres also, and are commonly referred to as the vagosympathetic trunks. To minimize sympathetic effects of nerve stimulation.

sufficient propranolol ($4 \mu\text{g ml}^{-1}$) was added to the perfusate to block β -adrenoreceptors (O'Donnell and Wanstall, 1982).

In another five toads, the temperature of the whole animal was altered. In these toads, therefore, no attempt was made to hold cardiac output constant. Toads were placed in a metal dish which was submerged in a controlled-temperature water bath. The body of the toad was immersed in paraffin oil, which served to keep nerves and tissues from drying out and helped to keep the animal at a constant temperature. A temperature probe, attached to a telethermometer (Yellow Springs Instrument Co. Inc.) was placed next to the heart, and temperature was recorded. Four of the toads were studied at two temperatures, and one toad was studied at six temperatures within the range $10\text{--}28^\circ\text{C}$. In these toads, the electrocardiogram (ECG) was recorded *via* two fine platinum wire electrodes, one inserted into the ventricular muscle and one in a pectoral muscle. The ECG signal triggered a Grass tachograph for recording beat-by-beat pulse interval, which was measured to the nearest 20 ms. Pulse interval and ECG were recorded continuously on a Grass polygraph. A moist stream of oxygen was bubbled slowly into the lungs *via* a cannula tied into the wall of the left lung. Gas escaped through the glottis, which was held open with a small piece of polyethylene catheter tubing. In this way, the lung gas remained oxygen-rich, and the blood remained oxygenated. β -Adrenoreceptor blockade was established by intravenous injection of propranolol (1 mg kg^{-1}) prior to recording.

In all toads, both vagus nerves were cut high in the neck under the angle of the jaw, above the point at which the nerve divides into laryngeal, cardiac, pulmonary and gastric branches. The peripheral end of the right vagus was stimulated using a pair of platinum electrodes. Trains of supramaximal square-wave pulses of 1 ms duration and a known frequency were delivered from an isolated square-wave stimulator. At each temperature, pulse interval was recorded prior to vagal stimulation (stimulation frequency = 0 Hz) and during vagal stimulation at three different frequencies, 0.5, 1 and 1.5 Hz. At each stimulation frequency, pulse interval increased gradually and reached a steady level, usually within 2 min even at low temperatures. At this stage, 10 consecutive pulse interval measurements were taken. Pulse interval was allowed to return to pre-stimulation levels before the next frequency was applied.

At each temperature, for each animal, the relationship between pulse interval and vagal frequency was assessed by linear regression analysis. The proportion of the total variation in pulse interval accounted for by the fitted regression line was calculated as the coefficient of determination, r^2 , which is a measure of the strength of the straight-line relationship (Zar, 1984).

For the five toads in which the hearts were perfused from a 'constant-pressure reservoir', the slopes of the regression lines relating pulse interval to stimulation frequency were grouped into four temperature ranges and compared by analysis of variance. The mean slope for each temperature range was then compared with all other ranges by a Newman-Keuls test (see Zar, 1984).

Prior to experiments, all toads had been kept for at least 1 month at $20\text{--}24^\circ\text{C}$.

Results

When the peripheral end of the vagus nerve is stimulated in the toad, the heart slows. As frequency of vagal stimulation increases, a linear increase in the pulse interval is recorded (Fig. 1A). Thirty-two linear regression lines from all 10 animals were fitted to the data points relating pulse interval to vagal stimulation. All showed a significant positive slope. Thirty of the 32 lines had coefficients of determination (r^2) between 0.9 and 1.0. In the four animals studied with an intact circulation, in which the body temperature of the whole animal was changed, 12 regression lines were fitted. All had coefficients of determination greater than 0.98. These high r^2 values indicate that a straight line describes the relationship well.

The slope of the linear relationship between pulse interval and vagal frequency can define the effectiveness of the vagus in slowing the heart (Parker *et al.* 1984). The effectiveness of the vagus in toads increases as temperature decreases. This is true whether the circulation is intact, or whether the heart is perfused with

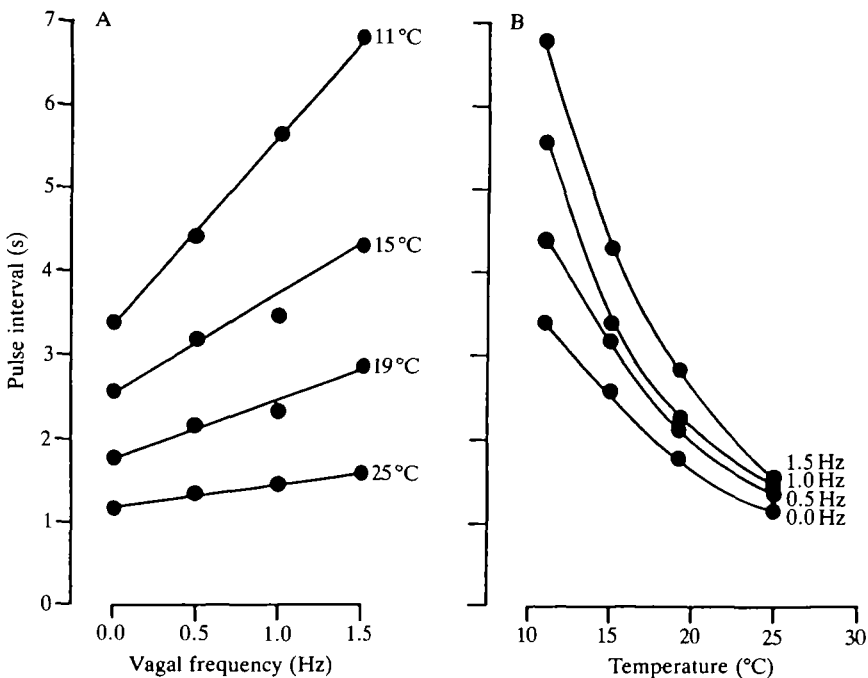


Fig. 1. (A) Relationship between pulse interval and frequency of vagal stimulation in one 'intact' toad at four different temperatures. The slope of each line is an indication of vagal effectiveness. As the toad was cooled, the same vagal stimulus slowed the heart more effectively. (B) Relationship between pulse interval and temperature for each of four stimulation frequencies. When the vagus was not stimulated (0 Hz), pulse interval increased spontaneously with decreasing temperature. The lower the temperature the greater the effect of increasing vagal stimulation frequency. For both A and B, the standard error bars for each point lie wholly within the circle designating each point.

Ringer's solution. In every toad, as temperature decreased, the spontaneous atrial rate decreased, and the effectiveness of the vagus on pulse interval increased (Fig. 1A). The same pulse interval data are plotted in Fig. 1B, but as a function of temperature. At low temperatures the stimulated pulse intervals are more divergent than at high temperatures, indicating that vagal stimulation is more effective at low temperatures.

Five toads were studied in which oxygenated Ringer's solution was perfused through the heart at a constant pressure, to give a constant flow. Under these conditions the vagus was more effective at low temperatures, indicating that the phenomenon is not dependent on changes of cardiac perfusion with temperature. Each toad was tested at four temperatures between 10 and 37°C. The linear regression lines relating pulse interval to vagal stimulation for each of the five toads over this temperature range are shown in Fig. 2. The data points for each toad are represented by the same individual symbol at each temperature. A

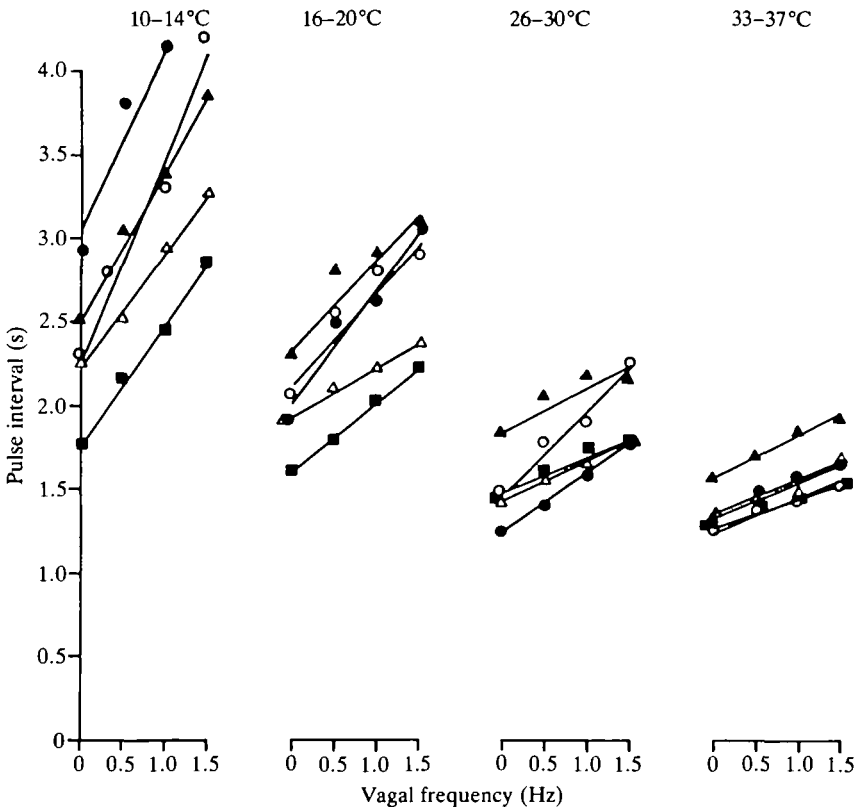


Fig. 2. Linear regression lines relating pulse interval to vagal stimulation for the five toads studied under conditions of controlled perfusion of their hearts. In each temperature category, one line represents data from one toad. All five toads are represented in each temperature category. Each toad can be identified by use of a consistent symbol to represent the data points on each line. As temperature increases, the effectiveness of the vagus decreases.

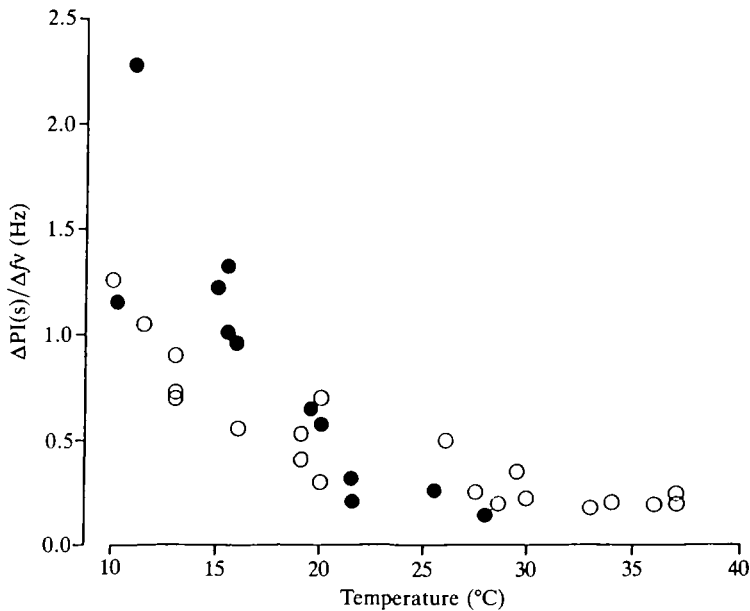


Fig. 3. The relationship between vagal effectiveness, determined by the slope of the line relating pulse interval (PI) to vagal stimulation frequency (f_v), and temperature in 'intact' toads (●) and 'perfused' toads (○). Each point represents a slope determined at one temperature, in one toad. The r^2 value for each slope is between 0.9 and 1 (see Results). At low temperatures, vagal effectiveness increases more markedly in 'intact' toads.

Newman-Keuls test comparing the mean vagal effectiveness for each of the four temperature groups showed that: mean 10–14°C ≠ mean 16–20°C = mean 26–30°C = mean 33–37°C. Thus, there is a hyperbolic relationship between the effectiveness of the vagus (as determined by the slope of the linear regression line) and temperature. This relationship holds for both groups of toads (Fig. 3). However, at low temperatures, the effectiveness of the vagus appears to be greater in the intact toads than in the toads with perfused hearts. If the effectiveness of the vagus ($\Delta\text{PI}/\Delta f_v$) is plotted against the inverse of temperature, the data for each group of toads separately are described by a straight line, and the slopes of the two lines are significantly different from each other ($t=2.88$, d.f.=22, $P<0.01$) (Table 1). In comparing these slopes, only data from the range of temperatures common to both groups were used (10–28°C), although all data points are plotted in Fig. 3. Thus, in all toads studied, temperature altered the effectiveness of the vagus, the effect being more marked in intact toads than in toads in which the heart was perfused at a constant pressure and flow.

Discussion

As toad body temperature is lowered, the cardiac vagus becomes more effective

Table 1. Linear regression data for the relationship between vagal effectiveness ($\Delta PI/\Delta fv$) and the inverse of temperature in two groups of toads (five 'intact' toads, five 'perfused' toads)

Toad group	Slope	y-intercept	Coefficient of determination	N (no. of slopes)
Intact	27.64 (s.e. 5.27)	-0.82	0.86	12
Perfused	13.30 (s.e. 1.74)	-0.18	0.83	14

The slopes of the lines are significantly different ($P < 0.01$).

For each group of toads, only data from the range of temperatures common to both groups were used (i.e. 10–28°C).

PI, pulse interval; fv , frequency of vagal stimulation.

at slowing the heart. The increase in vagal effectiveness causes much larger increases in pulse interval than the direct action of cold on the unstimulated heart. This phenomenon is similar to that described over a narrow temperature range in mammals (Potter *et al.* 1985). The mechanism by which this effect occurs has not been determined, but several factors could be operating either alone or together. For example, there could be changes in the output of transmitter from the nerve endings, in the sensitivity of the pacemaker to the transmitter, in the resting potential of the pacemaker cells or in the activity of cholinesterases.

It is not known how acetylcholine (ACh) release at the heart is affected by temperature. Although measurements have been made at the neuromuscular junction in various animals, it is difficult to extrapolate these results to the heart. At the neuromuscular junction in the frog, transmitter release increases between 10 and 20°C (Takeuchi, 1958), as it does in a mammal (Hubbard *et al.* 1971). However, Hubbard *et al.* (1971) made measurements over a broader temperature range (10–40°C) and they found a complex relationship with transmitter release peaking at 20°C and declining between 20 and 30°C. Transmitter release was found to peak in an antarctic fish also (this time at 5°C) and was followed by a decline until transmission ceased at 18°C (Pockett and Macdonald, 1986). Such complex patterns of transmitter release with temperature could not alone account for the results described here. The increased vagal effectiveness at low temperatures could be caused by a decline in cholinesterase activity (Takeuchi, 1958). Such a decline would allow a greater build-up of ACh and thus a more pronounced effect of the nerve at the pacemaker.

The availability, or receptivity, of α - and β -adrenoreceptors in the frog heart appears to alter with temperature (e.g. Buckley and Jordan, 1970; Caron and Lefkowitz, 1974; Benfey, 1975). No comparable studies have been carried out on muscarinic receptors, but a similar shift in receptor availability, if it were to occur, could contribute to the effect described here. Whatever the mechanism, the temperature dependence of vagal effectiveness has important physiological

consequences for an animal whose temperature varies. It emphasizes the importance of peripheral factors in considerations of nerve actions. The effect of decreasing temperature, for example, on peripheral nerve endings may cause significant amplification of neural traffic in many nerves.

There appears to be some vagal tone on the heart of amphibians during resting conditions (Lillo, 1979) which would be affected by this phenomenon. Of greater importance is the influence of temperature on the many cardiovascular and respiratory reflexes which rely on the vagus nerve to affect the heart (Jones, 1966; Lund and Dingle, 1968; West and Van Vliet, 1983). Such reflexes may be much more effective at low than at high temperatures. However, it is possible that the increased effectiveness of each vagal impulse may be counterbalanced by a decrease in the number of impulses arriving at the heart at cooler temperatures. The transmission properties of the nerve undoubtedly will change with decreasing temperature (Gasser, 1931; Takeuchi, 1958; Linden *et al.* 1981), and maximum firing rates may become limited. In the frog *Rana pipiens*, the vagus became ineffective at slowing the heart below 10°C (Young, 1959; Lund and Dingle, 1968). In the present study, the observation that the relationship between PI and vagal frequency of stimulation remained linear at all the temperatures studied suggested that nerve transmission was not impaired at the lower temperatures.

The difference in vagal effectiveness on the hearts of 'intact' toads compared with perfused hearts at low temperatures can most probably be explained in terms of cardiac output. While, in perfused preparations, the flow through the heart remained unaltered over a range of temperatures, cardiac output would have decreased as heart rate declined in the intact toads. Such a decline in cardiac output may allow for greater accumulation of ACh and thus a prolonged effect of the vagus on the heart (Courtice and McCloskey, 1985).

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References

- BENFEY, B. G. (1975). Temperature dependence of phenoxybenzamine effects and the adrenoceptor transformation hypothesis. *Nature, Lond.* **256**, 745–747.
- BUCKLEY, G. A. AND JORDAN, C. C. (1970). Temperature modulation of α - and β -adrenoceptors in the isolated frog heart. *Br. J. Pharmac.* **38**, 394–398.
- CARON, M. G. AND LEFKOWITZ, R. J. (1974). Temperature immutability of adenylyl cyclase-coupled β adrenergic receptors. *Nature, Lond.* **249**, 258–260.
- CLARK, A. J. (1920). The effect of alterations of temperature upon the functions of the isolated heart. *J. Physiol., Lond.* **54**, 275–286.
- COURTICE, G. P. (1985). Effect of hypoxia on cardiac vagal action in a lizard *Physignathus lesueurii*, and its contribution to diving bradycardia. In *Biology of Australasian Frogs and Reptiles* (ed. G. C. Grigg, R. Shine and H. Ehmann), pp. 373–377. Sydney: Surrey Beatty and Sons.
- COURTICE, G. P. AND MCCLOSKEY, D. I. (1985). Modification of vagal action on the toad heart by changes in flow. *J. comp. Physiol.* **155**, 571–575.

- COURTICE, G. P., POTTER, E. K. AND McCLOSKEY, D. I. (1983). Potentiation of the action of the vagus nerve on the heart in severe hypoxia. *J. auton. nerv. Syst.* **7**, 175–178.
- GASSER, H. S. (1931). Nerve activity as modified by temperature changes. *Am. J. Physiol.* **97**, 254–276.
- HUBBARD, J. I., JONES, S. F. AND LANDAU, E. M. (1971). The effect of temperature change upon transmitter release, facilitation and post-tetanic potentiation. *J. Physiol., Lond.* **216**, 591–609.
- JONES, D. R. (1966). Factors affecting the recovery from diving bradycardia in the frog. *J. exp. Biol.* **44**, 397–411.
- LILLO, R. S. (1979). Autonomic cardiovascular control during submergence and emergence in bullfrogs. *Am. J. Physiol.* **237**, R210–R216.
- LINDEN, R. J., MARY, D. A. S. G. AND WEATHERILL, D. (1981). The effect of cooling on the transmission of impulses in vagal nerve fibres attached to atrial receptors in the dog. *Q. Jl exp. Physiol.* **66**, 321–332.
- LUND, G. F. AND DINGLE, H. (1968). Seasonal temperature influence on vagal control of diving bradycardia in the frog (*Rana pipiens*). *J. exp. Biol.* **48**, 265–277.
- O'DONNELL, S. R. AND WANSTALL, J. C. (1982). Pharmacological experiments demonstrate that toad (*Bufo marinus*) atrial beta-adrenoceptors are not identical with mammalian beta₂- or beta₁-adrenoceptors. *Life Sci.* **31**, 701–708.
- PARKER, P., CELLER, B. G., POTTER, E. K. AND McCLOSKEY, D. I. (1984). Vagal stimulation and cardiac slowing. *J. auton. nerv. Syst.* **11**, 226–231.
- POCKETT, S. AND MACDONALD, J. A. (1986). Temperature dependence of neurotransmitter release in the antarctic fish *Pagothenia borchgrevinki*. *Experientia* **42**, 414–415.
- POTTER, E. K. (1982). Angiotensin inhibits action of vagus nerve at the heart. *Br. J. Pharmac.* **75**, 9–11.
- POTTER, E. K., McCLOSKEY, D. I. AND COURTICE, G. P. (1986). Effects of hypoxia, hypercapnia and acidemia on vagal action at the heart in the dog. *J. auton. nerv. Syst.* **16**, 79–83.
- POTTER, E. K., PARKER, P., CAINE, A. C. AND LUMBERS, E. R. (1985). Potentiation of cardiac vagal action by cold. *Clin. Sci.* **68**, 165–169.
- TAKEUCHI, N. (1958). The effect of temperature on the neuromuscular junction of the frog. *Jap. J. Physiol.* **8**, 391–404.
- VAN VLIET, B. N. AND WEST, N. H. (1987). Responses to circulatory pressures, and conduction velocity, of pulmocutaneous baroreceptors in *Bufo marinus*. *J. Physiol., Lond.* **388**, 41–53.
- WEST, N. H. AND VAN VLIET, B. N. (1983). Open-loop analysis of the pulmocutaneous baroreflex in the toad *Bufo marinus*. *Am. J. Physiol.* **245**, R642–R650.
- YOUNG, W. (1959). Temperature and vagal effects. *Am. J. Physiol.* **196**, 824–826.
- ZAR, J. H. (1984). *Biostatistical Analysis*, second edn. Englewood Cliffs, NJ: Prentice-Hall Inc.

