

## Control of heart rate during thermoregulation in the heliothermic lizard *Pogona barbata*: importance of cholinergic and adrenergic mechanisms

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### Summary

During thermoregulation in the bearded dragon *Pogona barbata*, heart rate when heating is significantly faster than when cooling at any given body temperature (heart rate hysteresis), resulting in faster rates of heating than cooling. However, the mechanisms that control heart rate during heating and cooling are unknown. The aim of this study was to test the hypothesis that changes in cholinergic and adrenergic tone on the heart are responsible for the heart rate hysteresis during heating and cooling in *P. barbata*. Heating and cooling trials were conducted before and after the administration of atropine, a muscarinic antagonist, and sotalol, a  $\beta$ -adrenergic antagonist. Cholinergic and  $\beta$ -adrenergic blockade did not abolish the heart rate hysteresis, as the heart rate during heating was significantly faster than during cooling in all cases. Adrenergic tone was extremely high (92.3%) at the

commencement of heating, and decreased to 30.7% at the end of the cooling period. Moreover, in four lizards there was an instantaneous drop in heart rate (up to 15 beats min<sup>-1</sup>) as the heat source was switched off, and this drop in heart rate coincided with either a drop in  $\beta$ -adrenergic tone or an increase in cholinergic tone. Rates of heating were significantly faster during the cholinergic blockade, and least with a combined cholinergic and  $\beta$ -adrenergic blockade. The results showed that cholinergic and  $\beta$ -adrenergic systems are not the only control mechanisms acting on the heart during heating and cooling, but they do have a significant effect on heart rate and on rates of heating and cooling.

Key words: thermoregulation, heart rate, neural control, cholinergic, adrenergic, reptile, lizard, *Pogona barbata*.

### Introduction

Heliothermic reptiles regulate their body temperature ( $T_b$ ) by behavioural means (Hertz et al., 1993; Seebacher, 1999), and it is well known that the effectiveness of behavioural thermoregulation can be augmented by changes in internal heat transfer brought about by modifications in heart rate and blood flow (Bartholomew and Tucker, 1963; Robertson and Smith, 1979; Grigg and Seebacher, 1999; Seebacher, 2000). In vertebrates other than fish, cardiac output is primarily determined by heart rate, rather than by changes in stroke volume (Farrell, 1991). Moreover, changes in heart rate have been shown to be a good indicator for changes in peripheral blood flow in several species of reptile (Morgareidge and White, 1972; Grigg and Alchin, 1976; Smith, 1976; Smith et al., 1978). For example, wash-out rates of radioactive Xe isotope injected under the skin increase dramatically with the application of heat, and decrease when the heat source is removed, indicating thermally dependent changes in peripheral blood flow that are accompanied by proportional changes in heart rate (Grigg and Alchin, 1976; Smith et al., 1978).

Several species of reptiles, including lizards, crocodylians and turtles, are known to increase their heart rate during basking, resulting in increased heat transfer between the warm

animal surface and the cool core (Bartholomew and Tucker, 1963; Grigg and Seebacher, 1999; Smith, 1976; Voigt, 1975). Conversely, when entering a cooling environment at high  $T_b$ , heart rate decreases so that heat transfer between the warm core and the cool surface decreases (Seebacher, 2000). This pattern, where heart rate during heating is significantly faster than during cooling, is termed heart rate hysteresis, and it allows a reptile to stay warm for longer during the day by raising the body temperature faster during basking in the morning and reducing the rate of cooling in the evening (Seebacher, 2000). Despite the functional significance of these changes in heart rate, the physiological mechanisms that effect changes in heart rate remain obscure.

The sympathetic (adrenergic) and the parasympathetic (cholinergic) nervous systems are principally responsible for short-term (on the scale of seconds or minutes) cardiovascular control in vertebrates (Akselrod et al., 1981). The cholinergic, vagal branch of the autonomic nervous system uses acetylcholine as a transmitter substance to depress heart rate by acting on heart muscarinic receptors. In contrast, spinal autonomic fibres effect an increase in heart rate, which is mediated by adrenaline acting on heart  $\beta$ -adrenergic receptors

(Axelsson et al., 1987; Morris and Nilsson, 1994). The autonomic fibres controlling the heart are continuously active, thereby creating a nervous tone which increases the efficacy of the heart rate response (Altimiras et al., 1997; Hoffman and Romero, 2000). There is evidence that changes in the cholinergic tone on the heart are the principle mechanism controlling heart rate during exercise in fish (Axelsson et al., 1987; Altimiras et al., 1997). In contrast, heart rate variability in a lizard (*Gallotia galloti*) appeared to be mediated primarily by  $\beta$ -adrenergic receptor mechanisms (DeVera et al., 2000). Moreover, there are species-specific differences in the relative importance of cholinergic and adrenergic autonomic control of the heart. For example, the heart of the toad *Bufo paracnemis* at rest was under the influence of both cholinergic and adrenergic tone (Hoffman and Romero, 2000), whereas heart rate in resting *Bufo marinus* was regulated by cholinergic fibres alone, but tachycardia during exercise in this species was effected by adrenergic fibres (Wahlqvist and Campbell, 1988).

Given the predominant role played by the autonomic nervous system in controlling heart rate of ectotherms, we postulated that autonomic neural mechanisms were responsible for heart rate regulation during body heating and cooling in the bearded dragon *Pogona barbata*, a heliothermic lizard. More specifically, we tested the hypothesis that changes in cholinergic and  $\beta$ -adrenergic tone on the heart are responsible for the heart rate hysteresis observed during heating and cooling in reptiles. Identification of the mechanism controlling heart rate during heating and cooling is of importance, because it will help us to understand how reptiles regulate their body temperature physiologically, and indicate on which physiological systems selection pressures may have acted to produce the thermoregulatory strategies seen in vertebrates today.

## Materials and methods

### *Experimental animals and set-up*

Seven bearded dragons (*Pogona barbata*) were hand-captured in south-east Queensland, Australia (24.4°S, 153.2°E) and transferred to outdoor cages at The University of Queensland in Brisbane, Australia. Animals were held for the duration of the experiments (2–3 weeks), after which they were released at their point of capture. Lizards were transferred to a constant temperature room (22.5°C) at least 24 h before experiments, so that body temperature  $T_b$  equalled ambient temperature at the start of experimentation. Heart rate signals from one individual were very weak and obscured by environmental noise, so that data from only six animals are presented here.

For the duration of the experiments, lizards were kept in plastic containers (30×37×28 cm), which were large enough for the animals to sit comfortably on the bottom without, however, allowing extensive movement. Electrocardiograms (ECGs) were measured with a high gain AC amplifier (BioAmp, AD Instruments, Powerlab frontend) that was connected to a 4-channel PowerLab (AD Instruments). The signals were sampled at 30 Hz by Chart software run on a Toshiba Laptop computer, which also calculated heart rates. Electrodes consisted of

insulated surgical stainless steel wire placed under the skin (after administration of Lignocaine as a local anaesthetic), one immediately ventral to the heart and a second at the base of the tail. The insulation was stripped off at the active ends of the electrodes, leaving approximately 1 cm of wire bared.  $T_b$  was measured with K-type thermocouples inserted 3–4 cm into the cloaca and also connected to the PowerLab.

During the experiment, lizards were heated from about 22.5°C to 32.5°C with an infrared heat lamp suspended above the plastic containers. The heat lamp was positioned at such a distance that the lizard surface received 600–700 kW m<sup>-2</sup>, which is similar to solar irradiation during basking on a summer morning (F. S., unpublished data; radiation intensity was measured with a Sol Data pyranometer connected to a datalogger). Once  $T_b$  reached 32°C the heat lamp was turned off and lizards were allowed to cool to within 1°C of their initial  $T_b$ . Heart rate and  $T_b$  were monitored continuously during the heating and cooling trials.

To control for potential effects of light, rather than heat, on heart rate, experimental trials were run with a cold, optical fibre light (Euromex) covered with red plastic foil instead of the infrared heat lamp.

### *Pharmacological protocol and treatments*

The effect of the autonomic nervous system on heart rate during heating and cooling was investigated by chemically blocking the  $\beta$ -adrenergic and muscarinic receptors. Atropine sulfate (1.5 mg kg<sup>-1</sup> body mass) was used as a muscarinic receptor (cholinergic) antagonist, and  $\beta$ -adrenergic receptors were blocked with the antagonist sotalol hydrochloride (3.0 mg kg<sup>-1</sup> body mass). Atropine and sotalol were dissolved in 0.9% saline and injected intraperitoneally. Saline solution was injected for control treatments.

The experiment consisted of four treatments: Control 1, heating and cooling after injection with saline solution; Control 2, cold red light after injection of saline solution; Treatment 1, heating and cooling after blocking the cholinergic nervous system by injecting atropine; Treatment 2, heating and cooling after administration of atropine and sotalol injected simultaneously. The pharmacological protocol follows details described by Altimiras et al. (1997). Lizards were injected 2 h before conducting heating and cooling trials to minimize the effect of handling stress. Blockade of the cholinergic and adrenergic systems was established from stabilization of heart rate, which typically occurred 30–60 min after injection of the antagonists. During each treatment, heart rate and  $T_b$  were monitored at least 5 min before the heat/cold lamp was switched on, and experimental equipment could be operated without disturbing the animals. As a rule, lizards sat quietly in the plastic container, but some of the animals moved occasionally, and this was clearly discernible on the ECG trace by the presence of electromyograms from skeletal muscular activity. These data were omitted from the analysis.

### *Analysis*

Changes in heart rate were analysed statistically by a three-

way analysis of variance (ANOVA) with heating/cooling, treatment (control, atropine, atropine and sotalol) and lizard (1–6) as factors. To overcome possible dependence of sequential measurements, heart rate measurements were randomized, and a random sub-sample of 15 measurements per level of each factor were used in the analysis. Cholinergic and  $\beta$ -adrenergic tones were calculated as follows (Altimiras et al., 1997):

$$\text{Cholinergic tone (\%)} = \frac{[\text{HR}_{\text{control}} - \text{HR}_{\text{chol}}]}{\text{HR}_{\text{complete}}} \times 100, \quad (1)$$

$$\text{Adrenergic tone (\%)} = \frac{[\text{HR}_{\text{complete}} - \text{HR}_{\text{chol}}]}{\text{HR}_{\text{complete}}} \times 100, \quad (2)$$

where  $\text{HR}_{\text{control}}$  is heart rate during control treatment,  $\text{HR}_{\text{chol}}$  is heart rate during cholinergic blockade (atropine treatment), and  $\text{HR}_{\text{complete}}$  is heart rate during complete autonomic blockade (atropine + sotalol).

Changes in  $\beta$ -adrenergic and cholinergic tone during heating and cooling were analysed by model 1 linear regression analysis with tone as the dependent variable and  $T_b$  as the independent variable. Data were presented in chronological order, but rather than plotting time on the  $x$ -axis, tone was plotted against  $T_b$  so that the problem of slightly different body masses and, therefore, different heating and cooling times of the study animals, was overcome.

Rates of heating and cooling were expressed as the transient rate of change in the internal temperature of the lizards. Body temperature was expressed as the dimensionless temperature  $\theta = (T_b - T_e)/(T_i - T_e)$ , where  $T_e$  is the operative temperature during the heating or cooling trial, and  $T_i$  is the initial body temperature of a lizard at the beginning of each heating or cooling episode (Seebacher, 2000). Rates of heating with the different treatments were compared by regressing  $\ln(\theta)$  over time for the heating trial of each lizard, and comparing the slopes of the regression by a one-way analysis of variance with treatment as factor.

## Results

There was a pronounced heart rate response to the application of heat under all experimental treatments and in all lizards (Fig. 1A–C, representative example from lizard 1). Heart rate increased after the heat lamp was switched on and, in the control treatment (Fig. 1A), it more than trebled (from 20 to 74  $\text{beats min}^{-1}$ ) during the  $10^\circ\text{C}$  increase in  $T_b$ . As soon as the heat lamp was switched off, heart rate dropped instantaneously by more than 10  $\text{beats min}^{-1}$  in the control treatment of lizard 1 (Fig. 1A). This drop in heart rate was apparent in the other study animals as well, and the mean instantaneous decrease in heart rate when lights were switched off was  $10.0 \pm 2.4$  (mean  $\pm$  S.E.M.)  $\text{beats min}^{-1}$ . The drop in heart rate at the moment the heat lamp was turned off was much less pronounced with a cholinergic blockade (Fig. 1B). The heart rate response to heat was much delayed with a total autonomic blockade (Fig. 1C), and the dramatic drop in heart rate seen in the control treatment was absent. Moreover, there was no response in heart rate when the control treatment was repeated

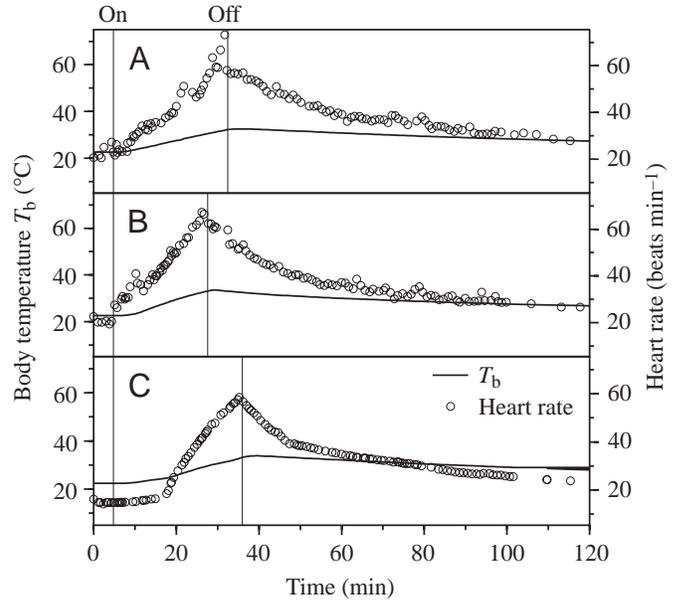


Fig. 1. Representative examples of heart rate (circles) and  $T_b$  (solid line) in *Pogona barbata* during heating and cooling. The first vertical line in each panel indicates when the infrared heat lamp was switched on, and the second vertical line indicates when the lamp was switched off. Lizards in the control treatment (A) were injected with saline, the muscarinic receptors were blocked during the atropine treatment (B) and cholinergic muscarinic and  $\beta$ -adrenergic receptors were blocked by injection of atropine+sotalol (C). All pharmaceuticals were administered 2 h before experimentation.

with a fibre optic, cold red light lamp, which confirms that lizards respond to heat rather than to light *per se* (Fig. 2).

Heart rate was significantly faster during heating than cooling in all lizards and for all treatments ( $F_{1,503}=1282.12$ ,  $P<0.0001$ ; Fig. 3), but heart rate varied significantly between lizards ( $F_{5,503}=1658.43$ ,  $P<0.001$ ) and between treatments

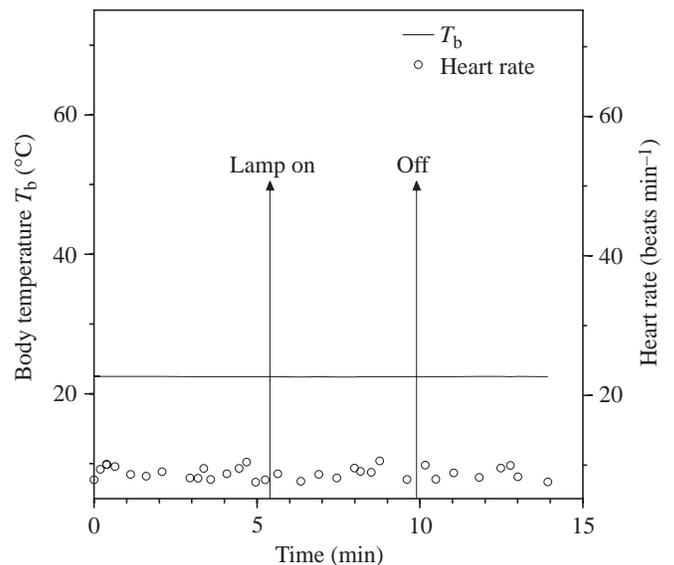


Fig. 2. Representative example of the cold red light control. Exposing lizards to cold red light had no effect on heart rate or  $T_b$ .

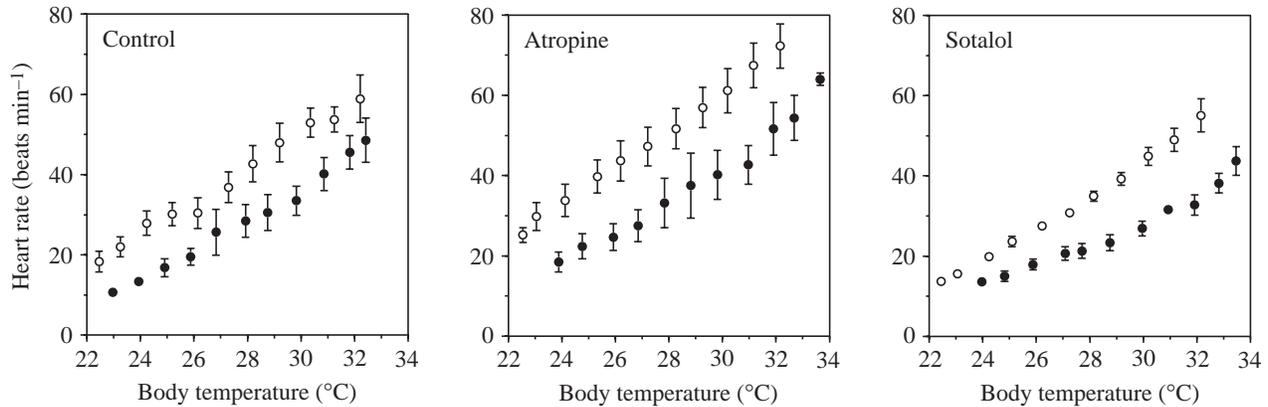


Fig. 3. Heart rates of all lizards during heating (open circles) and cooling (filled circles). Heart rate hysteresis was apparent during the control treatment (Control) as well as during the cholinergic blockade (Atropine) and the combined cholinergic +  $\beta$ -adrenergic blockade (Sotalol). Values are means  $\pm$  S.E.M. ( $N=6$ ).

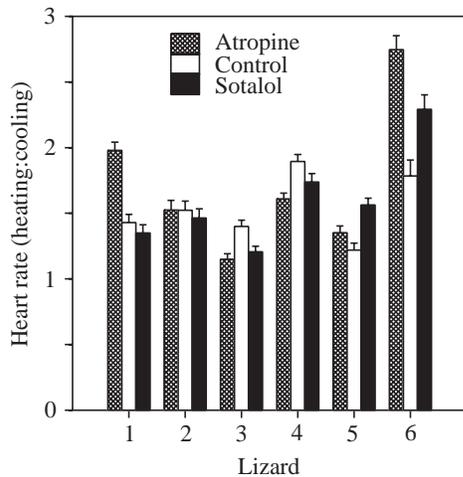


Fig. 4. The magnitude of the heart rate hysteresis during heating and cooling (expressed as the ratio of heart rate during heating:heart rate during cooling) was significantly different between lizards 1–6 and treatments (Atropine, cholinergic blockade; Control, control treatment; Sotalol, cholinergic +  $\beta$ -adrenergic blockade). For details, see text. Values are means  $\pm$  S.E.M.

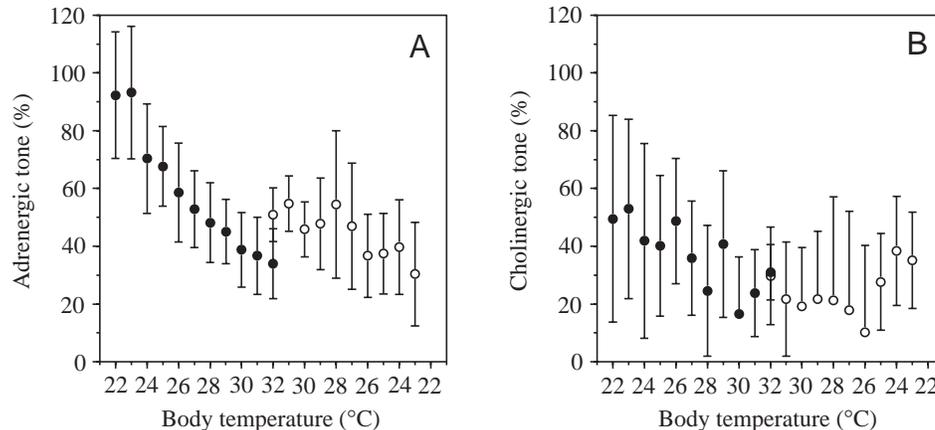


Fig. 5. Adrenergic (A) and cholinergic (B) tone on the heart during heating (filled circles) and cooling (open circles). Heart rate data were plotted against  $T_b$  during the heating and cooling trials so that the different body masses of the lizards were not confounding factors. Values are means  $\pm$  S.E.M.

( $F_{2,503}=387.09$ ,  $P<0.001$ ). Although blocking the cholinergic and  $\beta$ -adrenergic systems did not eliminate the heart rate hysteresis pattern, the different treatments did affect the magnitude of the hysteresis. Expressed as the ratio of heart rate during heating to heart rate during cooling (Fig. 4), the magnitude of heart rate hysteresis varied significantly between different treatments ( $F_{2,503}=46.58$ ,  $P<0.0001$ ), but the effect of the treatments also varied between lizards (Fig. 4). Cholinergic blockade significantly increased the magnitude of heart rate hysteresis in lizards 1 and 6 compared to controls, and combined cholinergic +  $\beta$ -adrenergic blockade increased the magnitude of the hysteresis in lizards 5 and 6. The ratio of heating to cooling heart rates was greatest in the control treatments of lizards 3 and 4.

Mean  $\beta$ -adrenergic tone on the heart was initially extremely high ( $>90\%$ ) during heating, and it decreased steadily as the animals continued to heat up (Fig. 5A;  $F_{1,10}=123.11$ ,  $P<0.0001$ ). Note, however, that immediately after the heat lamp was switched on,  $\beta$ -adrenergic tone varied between individuals, and it was as low as 10% in one lizard (see below, Fig. 6). During the cooling period, there was a slight increase in mean  $\beta$ -adrenergic tone as the heat lamp was turned off, but  $\beta$ -adrenergic tone decreased with decreasing  $T_b$ , to an overall low of 30.9% at the end of the cooling episode ( $F_{1,9}=19.33$ ,  $P<0.01$ ). Cholinergic tone was overall less than adrenergic tone during heating and cooling (Fig. 5B), but it was also greatest at the beginning of the heating episode and decreased as  $T_b$  increased ( $F_{1,10}=15.06$ ,  $P<0.01$ ). During cooling, cholinergic tone did not change, remaining at the low levels it reached at the end of the heating period ( $F_{1,9}=1.26$ ,  $P=0.29$ ).

Looking at a finer resolution of the tone on the heart during control

treatments of individual lizards around the time when the heat lamp was switched off (Fig. 6) reveals an interesting pattern. The  $\beta$ -adrenergic tone decreased sharply as the heat lamp was switched off in lizards 1 and 4 (Fig. 6A,D), while cholinergic tone increased at 'lamp off' in lizards 3 and 6 (Fig. 6C,F). No discernible patterns existed, however, in lizards 2 and 5 (Fig. 6B,E). Note that the negative cholinergic tone in lizard 3 (Fig. 6C) is due to heart rate during cholinergic blockade being greater than during control. This pattern indicates a very low cholinergic tone during the final part of the heating phase so that intra-individual variation in this particular lizard masks the effect, if there is any, of the cholinergic branch on heart rate.

Rates of heating were significantly different between the different treatments ( $F_{2,15}=4.55$ ,  $P<0.03$ ; Fig. 7) indicating that the autonomic nervous system may play a role in thermoregulation. Lizards heated faster with a cholinergic blockade than under control conditions, and rates of heating decreased when the autonomic nervous system was totally blocked (Fig. 7).

### Discussion

Control by the cholinergic and  $\beta$ -adrenergic control systems does not account for the heart rate hysteresis pattern in *P. barbata*, and heart rate during heating remained significantly faster than heart rate during cooling at any  $T_b$  following injection of atropine and sotalol. Nonetheless, there was an autonomic response to heat, and the influence of both the cholinergic and  $\beta$ -adrenergic neural control systems on heart rate had a significant effect on rates of heating. Lizards heated most rapidly in the presence of a cholinergic blockade, and slowest when  $\beta$ -adrenergic pathways were concurrently blocked. This is in agreement with the finding that cholinergic tone is relatively high during heating, and that adrenergic tone is extremely high during heating. The  $\beta$ -adrenergic branch of the autonomic nervous system significantly increased heart rate, and high  $\beta$ -adrenergic tone during heating could be expected if the lizard attempted to increase rates of heating.

Considering mean values from all lizards, it would appear that heart rate of *P. barbata* is regulated primarily by variation of the  $\beta$ -adrenergic tone on the heart. This is in contrast to fish, where heart rate during exercise is regulated primarily by variation in the cholinergic tone on the heart (Axelsson, 1988; Altimiras et al., 1997; Axelsson et al., 2001). Moreover, antarctic fish were found to

increase the cholinergic tone on their heart when heated, counteracting a temperature-induced increase in heart rate ( $Q_{10}$  effect), so that heart rate was thermally independent (Franklin et al., 2000). Care has to be taken, however, in drawing the conclusion that heart rate in *P. barbata* is primarily controlled by  $\beta$ -adrenergic receptors, because we found significant differences between individual lizards.

All lizards responded with a sudden drop in heart rate as the heat lamp was switched off, and this response appears to be a cholinergically mediated reflex as it disappears with the administration of atropine. The extremely rapid response to the removal of the heat source could indicate that thermal sensors

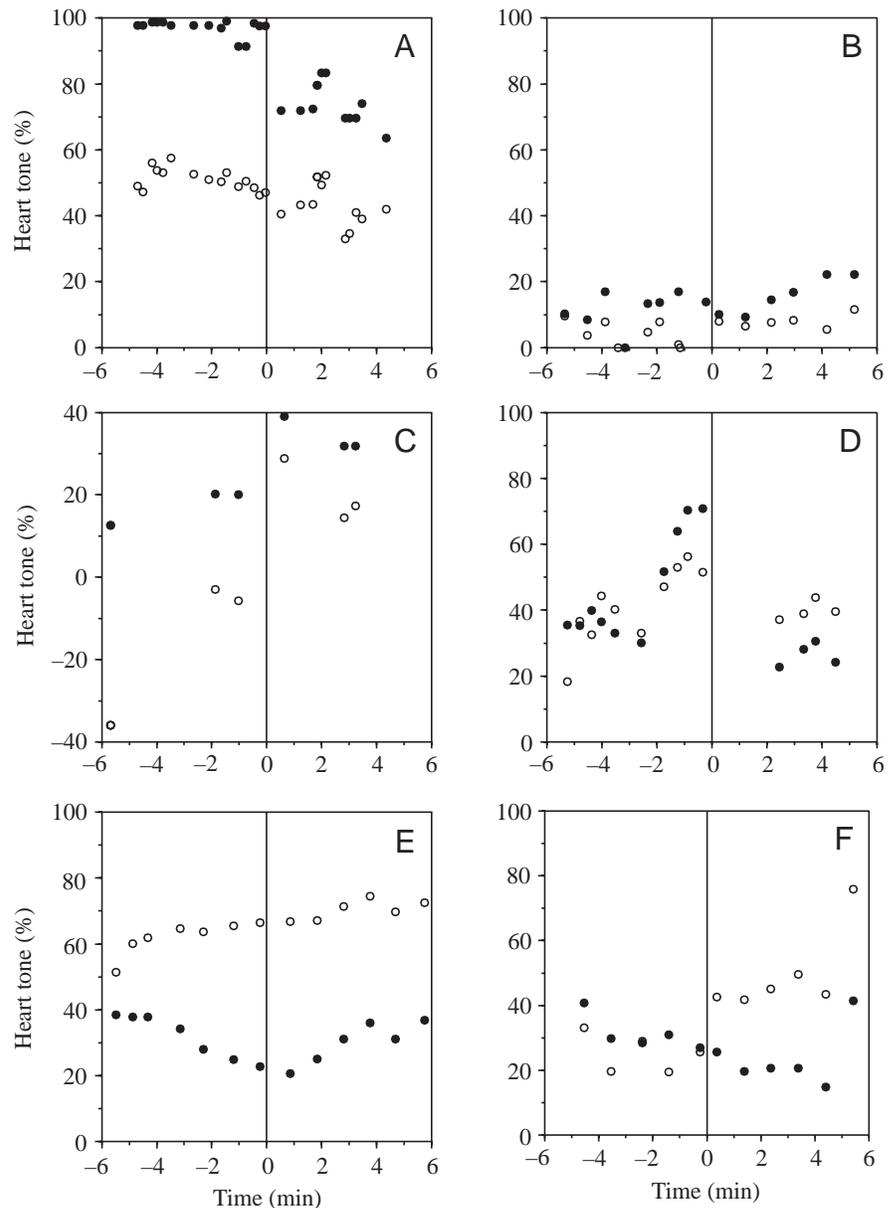


Fig. 6. The tone on the heart of individual lizards (A–F) several minutes before and after the heat lamp was switched off at the end of the heating episode. The time at which the lamp was switched off is indicated by the vertical line at 0 min on the x-axis. Filled circles, adrenergic tone; open circles, cholinergic tone.

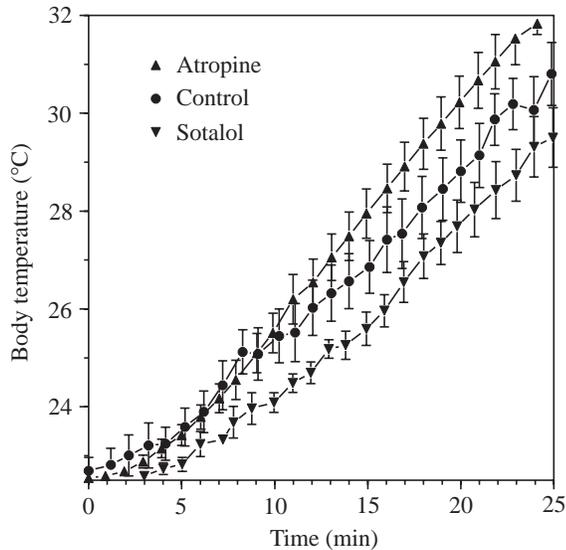


Fig. 7. Lizards heated fastest when the cholinergic branch was blocked (Atropine) and slowest when both the cholinergic and  $\beta$ -adrenergic branches were blocked (Sotalol). Values are means  $\pm$  S.E.M.

in the skin may instigate a cholinergic response *via* the action of prostaglandins, for example (Robleto and Herman, 1988). The existence of peripheral control mechanisms is also indicated by the fact that the increase in wash-out rates of radioactive Xe may precede an increase in heart rate after application of heat to the skin surface of the marine iguana (Morgareidge and White, 1972).

In exercising fish, cholinergic tone decreased by from 38% to 15% and adrenergic tone increased from 21% to 28% compared to resting values (Axelsson et al., 1987). Lizards in this study showed much greater variability in tone on the heart in response to heating and cooling but, again, there were pronounced differences between individuals. The  $\beta$ -adrenergic tone decreased by over 60%, and the cholinergic tone by over 40% between heating and cooling, so it must be concluded that there is a pronounced response to heating and cooling. It seems contradictory, however, that both  $\beta$ -adrenergic and cholinergic tones changed in the same direction and were lower during cooling than during heating. Akin to the fish example quoted above, we expected that as  $\beta$ -adrenergic tone increased, cholinergic tone would decrease, and *vice versa*, so that the total neural effect on heart rate is compounded. This was not the case, and it appears that the cholinergic and  $\beta$ -adrenergic branches work against each other. This relationship is also seen in the individual short-term responses to the sudden removal of the heat source where, if there is a change, cholinergic and  $\beta$ -adrenergic tone tend to change in the same direction.

Does an autonomic neural mechanism of thermoregulation exist in *P. barbata*? The cholinergic and  $\beta$ -adrenergic control systems certainly have an impact on heart rate, which would influence rates of internal heat transfer (Seebacher, 2000). However, in *P. barbata* these neural pathways are not responsible for the major cardiovascular mechanism in thermoregulation, i.e. the heart rate hysteresis pattern. It

appears, therefore, that there are other regulatory mechanisms controlling heart rate during heating and cooling and these may be more important in thermoregulation.

## References

- Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Barger, A. C. and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* **213**, 220–222.
- Altimiras, J., Aissaoui, A., Tort, L. and Axelsson, M. (1997). Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? *Comp. Biochem. Physiol.* **118A**, 131–139.
- Axelsson, M., Ehrenstrom, F. and Nilsson, S. (1987). Blood pressure regulation during exercise in the Atlantic cod *Gadus morhua*. *J. Exp. Biol.* **126**, 225–236.
- Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. Exp. Biol.* **137**, 287–303.
- Axelsson, M., Davison, W. and Franklin, C. E. (2000). Cholinergic and adrenergic tone on the heart of the Antarctic dragonfish, *Gymnodraco acuticeps*, living at sub-zero temperatures. *Exp. Biol. Online* **5:3**.
- Bartholomew, G. A. and Tucker, V. A. (1963). Control of changes in body temperature, metabolism, and circulation by the agamid lizard, *Amphibolurus barbatus*. *Physiol. Zool.* **37**, 199–218.
- DeVera, L., Gonzalez, J. and Pereda, E. (2000). Relationship between cortical electrical and cardiac autonomic activities in the awake lizard, *Gallotia galloti*. *J. Exp. Zool.* **287**, 21–28.
- Farrell, A. P. (1991). From hagfish to tuna: a perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137–1164.
- Franklin, C. E., Axelsson, M. and Davison, W. (2001). Constancy and control of heart rate during an increase in temperature in the Antarctic fish, *Pagothenia borchgrevinki*. *Exp. Biol. Online* **6:1**.
- Grigg, G. C. and Alchin, J. (1976). The role of the cardiovascular system in thermoregulation of *Crocodylus johnstoni*. *Physiol. Zool.* **49**, 24–36.
- Grigg, G. C. and Seebacher, F. (1999). Field test of a paradigm: hysteresis of heart rate in thermoregulation by a free-ranging lizard (*Pogona barbata*). *Proc. Roy. Soc. Lond. B* **265**, 1793–1799.
- Hertz, P. E., Huey, R. B. and Stevenson, R. D. (1993). Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**, 796–818.
- Hoffman, A. and Romero, S. M. B. (2000). Effect of the Dry-Cold season dormancy on the tonic and phasic neural control of heart rate in the toad, *Bufo paracnemis*. *J. Exp. Zool.* **287**, 15–20.
- Morgareidge, K. R. and White, F. N. (1972). Cutaneous vascular changes during heating and cooling in the Galapagos marine iguana. *Nature* **233**, 587–591.
- Morris, J. L. and Nilsson, S. (1994). The circulatory system. In *Comparative Physiology and Evolution of the Autonomic Nervous System* (ed. S. Nilsson and S. Holmgren), pp. 193–256. Chur, Switzerland: Harwood Academic Publishers.
- Robertson, S. and Smith, E. N. (1979). Thermal indications of cutaneous blood flow in the American alligator. *Comp. Biochem. Physiol.* **62A**, 569–572.
- Robleto, D. O. and Herman, C. A. (1988). Cardiovascular effects of prostaglandin I<sub>2</sub> and prostaglandin F<sub>2 $\alpha$</sub>  in the unanesthetized bullfrog *Rana catesbeiana*. *J. Exp. Zool.* **246**, 10–16.
- Smith, E. N. (1976). Heating and cooling rates of the American alligator, *Alligator mississippiensis*. *Physiol. Zool.* **49**, 37–48.
- Smith, E. N., Robertson, S. and Davies, D. G. (1978). Cutaneous blood flow during heating and cooling in the American alligator. *Am. J. Physiol.* **235**, R160–R167.
- Seebacher, F. (1999). Behavioural postures and the rate of body temperature change in wild freshwater crocodiles, *Crocodylus johnstoni*. *Physiol. Biochem. Zool.* **72**, 57–63.
- Seebacher, F. (2000). Heat transfer in a microvascular network: the effect of heart rate on heating and cooling in reptiles (*Pogona barbata* and *Varanus varius*). *J. theor. Biol.* **203**, 97–109.
- Voigt, W. G. (1975). Heating and cooling rates and their effects upon heart rate and subcutaneous temperatures in the desert tortoise, *Gopherus agassizii*. *Comp. Biochem. Physiol.* **52A**, 527–531.
- Wahlqvist, I. and Campbell, G. (1988). Autonomic influences on heart rate and blood pressure in the toad, *Bufo marinus*, at rest and during exercise. *J. Exp. Biol.* **134**, 377–396.