Prostaglandins are important in thermoregulation of a reptile (*Pogona vitticeps*)

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The effectiveness of behavioural thermoregulation in reptiles is amplified by cardiovascular responses, particularly by differential rates of heart beat in response to heating and cooling (heart-rate hysteresis). Heart-rate hysteresis is ecologically important in most lineages of ectothermic reptile, and we demonstrate that heart-rate hysteresis in the lizard *Pogona vitticeps* is mediated by prostaglandins. In a control treatment (administration of saline), heart rates during heating were significantly faster than during cooling at any given body temperature. When cyclooxygenase 1 and 2 enzymes were inhibited, heart rates during heating were not significantly different from those during cooling. Administration of agonists showed that thromboxane B₂ did not have a significant effect on heart rate, but prostacyclin and prostaglandin F_{2α} caused a significant increase (3.5 and 13.6 beats min⁻¹, respectively) in heart rate compared with control treatments. We speculate that heart-rate hysteresis evolved as a thermoregulatory mechanism that may ultimately be controlled by neurally induced stimulation of nitric oxide production, or maybe via photolytically induced production of vitamin D.

Keywords: thermoregulation; heart rate; prostaglandins; hysteresis; reptiles

1. INTRODUCTION

Unlike endothermic mammals and birds, ectothermic reptiles often rely entirely on behavioural means to regulate body temperatures. Importantly, reptiles can control rates of transient heat transfer between the environment and their core (O'Connor 1999; Seebacher 2000) by altering heart rates and peripheral circulation (Bartholomew & Tucker 1963; Morgareidge & White 1972). Accordingly, heart-rate increases during heating, primarily during basking (Grigg & Seebacher 1999), and decreases during cooling, such that the heart rate is significantly higher during heating than during cooling, a phenomenon known as heart-rate hysteresis (Grigg *et al.* 1979). Accelerated heart rates increase rates of heating, and the slowing of heart rates decreases rates of cooling (Grigg *et al.* 1979). The

ecological significance of heart-rate hysteresis for reptiles lies in decreasing the behavioural cost of thermoregulation and allowing body temperatures to be within preferred thermal ranges for significantly longer periods during the day (Seebacher 2000).

The physiological mechanisms controlling thermoregulation in reptiles are not well known. The light-sensitive pineal gland and the hypothalamus may function as a thermostat and direct thermally inspired behaviour (Tosini *et al.* 2001). Additionally, melatonin may influence behaviour and, hence, body temperature of diurnal reptiles (Lutterschmidt *et al.* 2002). The autonomic nervous system plays a part in mediating the acute response of heart rate to the application or removal of heat, but cholinergic and β -adrenergic mechanisms did not explain the pronounced differences between heart rates during heating and cooling (Seebacher & Franklin 2001). Experimental data (Morgareidge & White 1972) indicated that peripheral blood flow changed immediately before changes in heart rate. Changes in peripheral flow, which may be brought about by vasoactive substances such as prostaglandins, alter blood pressure and may affect heart rate via the barostatic reflex (Altimiras *et al.* 1998). Prostaglandins, which are formed from arachidonic acid by cyclooxygenase (COX) enzymes (Chandrasekharan *et al.* 2002), are local mediators that are implicated in many physiological functions of vertebrates, and thromboxane, prostacyclin I₂ and prostaglandin F_{2α} have been shown to be vasoactive in an amphibian (Robleto & Herman 1988). Hence, we tested the hypothesis that in reptiles cardiac responses during thermoregulation are controlled in peripheral vascular endothelia by the release of prostaglandins in response to changes in heat load at the animal surface (Franklin & Seebacher 2003).

2. MATERIAL AND METHODS

Bearded dragons (*Pogona vitticeps*; $n = 11$; mean mass $\pm$ s.e. = 288.4 ± 21.5 g) were collected in NSW, Australia (149.28 E, 31.28 S), and maintained in outdoor enclosures at the University of Sydney. Lizards were starved for 24 h prior to experimentation. Six animals were used for laboratory heating and cooling trials in which either saline (control) or the non-steroidal anti-inflammatory drug Diclofenac (Sigma; 8 mg kg⁻¹ body mass), an inhibitor of COX activity (Rainsford 2001), were injected intraperitoneally. After injection, animals were left undisturbed for 2.5 h before experimentation. During experimentation, animals were held unrestrained in plastic containers, which were large enough for the animals to sit comfortably but with limited movement. Heart rates were measured with temporary cardiac pacing wire electrodes (Medtronic, France) connected to a computerized data acquisition system (PowerLab, AD Instruments, Australia) fronted by a high-gain AC amplifier (BioAmp, AD Instruments, Australia). Electrodes were sutured in place under the skin ventral and dorsal to the heart. Body temperatures were measured by K-type thermocouples inserted 40–50 mm into the lizards' cloaca and connected to a thermocouple pod (AD Instruments, Australia) that was linked to the PowerLab. All monitoring equipment was controlled from a separate room adjoining the experimental chamber so that lizards were not disturbed during data collection. Heating and cooling trials were conducted during the day after implantation of the ECG electrodes and thermocouple.

Animals were heated from 21 °C to 30 °C with a 300 W heat lamp suspended above the plastic containers and allowed to cool convectively in a temperature-controlled room set at 21 °C. Heart rate and body temperature were measured continuously during heating and cooling. Heart-rate data were analysed by an analysis of covariance, with body temperature as the covariate.

The effect of prostaglandin agonists on heart rate was tested by injecting thromboxane B₂, prostacyclin, prostaglandin F_{2α} (ICN Chemicals; 10 μ g kg⁻¹; Robleto & Herman 1988) and saline into the caudal arteries of six lizards. Prostaglandins were dissolved in ethanol to make stock solutions of 1 mg ml⁻¹, which were diluted with 0.9% saline to make up the required concentration. Agonists were administered sequentially, and a saline control was conducted before and

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Figure 1. Representative examples of heart-rate (bold solid lines) responses of *Pogona vitticeps* during heating and cooling (solid lines: body temperature) after intraperitoneal injection with saline (control) or the COX 1 and 2 inhibitor Diclofenac (inhibitor). (a) Control: heart rate increased rapidly with the application of heat (broken vertical line), and it dropped instantaneously by 40 beats min^{-1} when the heat lamp was switched off (solid vertical line). (b) Control: heart rate during heating (open circles) was significantly faster than during cooling (filled circles) at any body temperature. (c) Inhibitor: heart rate increased proportionally to body temperature, but there were no differences in the rate of change between heating and cooling (heat on: broken vertical line; heat off: solid vertical line). (d) Inhibitor: there were no significant differences between heart rate during heating (open circles) and cooling (filled circles) at any temperature.

after each agonist treatment; the concentration of ethanol injected was too diluted to have a physiological effect (Crashaw *et al.* 1998). In all controls, the same quantity of saline was injected as drugs in experimental treatments. Note that some animals were used in both inhibitor and agonist experiments, but where animals were used repeatedly, experiments were conducted at least 7 days apart, and agonist treatments were conducted after the heating and cooling trials. Statistical significance was determined by comparing agonist treatments with control using two-sample *t*-tests.

3. RESULTS

In the control, heart rates increased dramatically with the application of radiant heat (figure 1a), and heart rates plummeted when the heat source was removed (figure 1a) despite the fact that body temperature continued to increase. Heart rates during cooling were significantly lower than during heating at any body temperature, showing a typical hysteresis pattern (figure 1b; ANCOVA on data from all study animals, $F_{1,97} = 45.3$, $p < 0.0001$). On average (means from all lizards), heart rates increased with increasing body temperature according to $Y = -106.40 + 6.22x$ ($R^2 = 0.99$) during heating, and $Y = -86.56 + 4.61x$ ($R^2 = 0.98$) during cooling. Heart-rate hysteresis disappeared completely when COX enzymes were inhibited. As expected from the Q_{10} effect, heart rates increased with increasing body temperature in animals treated with Diclofenac (figure 1c), but the rates of change were similar (mean: $Y = -63.33 + 4.64x$ ($R^2 = 0.96$)) and

there were no differences in heart rate between heating and cooling at any body temperature (figure 1d; ANCOVA, $F_{1,89} = 3.60$, $p > 0.05$). On average, in the control treatments heart rates during heating were about twice as high as during cooling, but the ratio between heating and cooling heart rates was close to unity when prostaglandin production was inhibited (figure 2).

There were no differences in heart rates between controls conducted before and after agonist treatments ($t = -0.38$, $p = 0.72$, d.f. = 5). Administration of thromboxane B_2 did not significantly change heart rate compared with controls ($t = -1.39$, $p = 0.22$; figure 3), but heart rates increased significantly after injection of prostacyclin (PGI_2 ; $t = -3.02$, $p < 0.03$) and prostaglandin $F_{2\alpha}$ ($t = -5.52$, $p < 0.01$; figure 3).

4. DISCUSSION

The heart-rate hysteresis response consists of two phases: an acute (less than 1 min) increase or decrease immediately following the application or removal of heat, respectively, which occurs before any change in body temperature. This is followed by a more prolonged phase, while the animal is heating or cooling, and during this time heart rate is significantly faster during heating than during cooling (Seebacher & Franklin 2001; Franklin & Seebacher 2003). The instantaneous response of heart rate to radiant heat is

Figure 2. The ratio of heart rates (means $\pm$ s.e.), during heating and cooling at different body temperatures were significantly greater during the control (open circles) than during the inhibitor treatment in which all ratios were close to unity (filled circles).

Figure 3. Heart rates (mean $\pm$ s.e.), did not change significantly after injection of thromboxane B₂ (TXB₂), but there were significant changes following administration of prostacyclin (PGI₂) and prostaglandin F_{2α} (PGF_{2α}).

controlled in part by β -adrenergic and cholinergic receptors (Seebacher & Franklin 2001), but we have shown here that prostaglandins are the major control agent of both phases. Prostaglandins act mainly in constricting or dilating blood vessels so that their effect on heart rate occurs most probably via a barostatic reflex in response to peripheral constriction or dilation of blood vessels. Thromboxane and prostacyclin are primarily produced in blood platelets and vascular endothelial cells, whereas prostaglandin F_{2α} is also formed in the heart muscle itself (Voet & Voet 1995), and there may be a direct effect on the heart in addition to that resulting from blood pressure changes. Prostacyclin and prostaglandin F_{2α} were shown to modulate heart rate and are, therefore, likely to influence heart rate during heating and cooling. Thromboxane B₂ had no effect at the concentration at which it was administered. It may be that thromboxane is active at higher concentrations in *P. vitticeps*, and it cannot be excluded as a potential control agent of heart rate during heating and cooling.

Induction of COX activity resulting in prostaglandin synthesis is the most likely cause explaining heart-rate regulation in response to heat. Activity of COX enzymes is induced by nitric oxide (NO; Salvemini *et al.* 1993), which is synthesized from L-arginine by NO synthase in endothelial cells. NO synthase activity is regulated by Ca²⁺ concentrations (Weaver *et al.* 2002), which may be altered by extracellular stimuli such as acetylcholine and epinephrine via the phosphoinositide cascade (Voet & Voet 1995; Wang *et al.* 2002). We speculate that neural signals from external thermoreceptors (Cesare & McNaughton 1996) may be responsible for the acute response of heart rate by controlling prostaglandin activity via Ca²⁺-induced NO production and COX activation. An alternative, but less likely scenario is that photolytically formed vitamin D stimulates release of Ca²⁺ from bone and other tissues (Langston *et al.* 1990). Hence, exposure to short-wave radiation may have an effect on heart rate by Ca²⁺-induced NO production and COX activity. This pathway may, however, be too slow to effect the observed changes in heart rate. In any case, these pathways of thermoregulatory control are fundamentally different to the centrally controlled thermostat and circadian regulators known from many vertebrates.

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