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Physiological importance of the coronary arterial blood supply to the rattlesnake heart

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SUMMARY

The reptilian heart consists of a thick inner spongy myocardium that derives its oxygen and nutrient supply directly from the blood within the ventricular cavity, which is surrounded by a thin outer compact layer supplied by coronary arteries. The functional importance of these coronary arteries remains unknown. In the present study we investigate the effects of permanent coronary artery occlusion in the South American rattlesnake (*Crotalus durissus*) on the ability to maintain heart rate and blood pressure at rest and during short term activity. We used colored silicone rubber (Microfil) to identify the coronary artery distribution and interarterial anastomoses. The coronary circulation was occluded and the snakes were then kept for 4 days at 30°C. Microfil injections verified that virtually all coronary arteries had successfully been occluded, but also made visible an extensive coronary supply to the outer compact layer in untreated snakes. Electrocardiogram (ECG), blood pressure (P_{sys}) and heart rate (f_H) were measured at rest and during enforced activity at day 1 and 4. Four days after occlusion of the coronary circulation, the snakes could still maintain a P_{sys} and f_H of 5.2±0.2 kPa and 58.2±2.2 beats min^{-1}, respectively, during activity and the ECG was not affected. This was not different from sham-operated snakes. Thus, while the outer compact layer of the rattlesnake heart clearly has an extensive coronary supply, rattlesnakes sustain a high blood pressure and heart rate during activity without coronary artery blood supply.

Key words: reptile, coronary artery occlusion, blood pressure, heart rate, Microfil.

INTRODUCTION

The ventricle of most ectothermic vertebrate hearts consists of an inner spongy myocardium surrounded by a thin outer compact layer (MacKinnon and Heatwole, 1981; Helle, 1983; Tota, 1983; Farrell et al., 1998; Ostadal et al., 1999). The spongious part of the myocardium is lost during the embryological development in mammals and birds, and the adult heart of the endothermic vertebrates, therefore, consists entirely of compact muscle. This morphological transition is probably correlated with increase in heart rate and blood pressure that attended the rise in metabolism with the evolution of endothermy (Tota, 1983; Burggren et al., 1997). The compact myocardium is often perfused with coronary arteries, whereas the spongy layer is avascular and depends entirely on oxygen diffusing from the lumen (Ostadal et al., 1999). Thus, the inner spongy myocardium consists of a network of intramyocardial channels and sinusoids providing a large endocardial surface area for gas and nutrient exchange, and the thin spongy network only represents a small diffusion distance between the ventricular chamber and the deepest muscle cells (Roethy et al., 1999).

The general form and structure of the coronary arteries have been described in representatives of most of the major taxonomic groups of reptiles (Grant and Regnier, 1926; Mackinnon and Heatwole, 1981; Farrell et al., 1998). In most species, the coronary artery arises at the root of the right aortic arch and bifurcates to ventral and dorsal branches that perfuse most of the ventricle (Grant and Regnier, 1926; Erhart, 1935; Mackinnon and Heatwole, 1981; Farrell et al., 1998). Very little, however, is known about the functional significance of the coronary blood supply to the ventricle in reptiles and its physiological role has not been addressed experimentally in any species of reptiles. The hearts of many reptiles is generally very hypoxia tolerant (e.g. Bing et al., 1972; Poupa et al., 1978; Overgaard et al., 2007), but force production of isolated ventricular strips of some snakes is markedly sensitive to hypoxia (Zaar et al., 2007). In turtles, hypoxia can affect the electrocardiogram, particularly during exercise when cardiac output is elevated and when the oxygen concentration of the venous blood is reduced (Farmer and Hicks, 2002).

Here we describe the coronary circulation of the South American rattlesnakes (*Crotalus durissus*). The cardiovascular physiology of this species of snake has been studied extensively (e.g. Galli et al., 2005; Skals et al., 2005; Skovgaard et al., 2005). To evaluate the importance of the coronary circulation, we compare the heart rate, blood pressure and electrocardiograms (ECGs) of snakes with normal, and snakes with occluded coronary circulation at rest and during enforced activity under the hypothesis that lack of coronary supply should impair the ability to maintain cardiac work.

MATERIALS AND METHODS

Experimental animals

Ten South American rattlesnakes (*Crotalus durissus* L.) of both sexes with a body mass of 620–945 g (764±42 g, mean ± s.e.m., *N*=10) were obtained from the Butantan Institute in São Paulo, Brazil. At the Jacarezario (UNESP, Rio Claro, Brazil), these snakes

were kept in individual chambers (0.5 m×0.5 m) at 28±5°C on a natural light regime. They always had free access to water and were fed on rodents and chickens no less than 7 days before surgery. All animals appeared healthy and experiments were performed in accordance with guidelines for animal experiments under Universidade Estadual Paulista (UNESP), Rio Claro, Brazil.

Surgery and instrumentation

Snakes were anaesthetised by halothane (5%) inhalation. When the animals no longer responded to tactile stimuli, local anaesthesia (Lidocaine, 1%) was injected and a 5 cm ventral incision was made cranial to the heart, so the pericardium could be opened to expose the heart. In six snakes, the coronary arteries were occluded by burning close to the outflow from the right aortic arch. Then, the pericardium was closed with two or three sutures (4–0 silk), and the cranial branch of the vertebral artery occlusively cannulated by insertion of a PE-50 catheter containing heparinized saline (50 i.u. ml^{-1}). This catheter was used for measurements of systemic arterial blood pressure (P_{sys}). The catheter was externalised and secured to the skin by several sutures. Finally, three ECG leads, constructed from the ends of 25 gauge hypodermic needles (Terumo, Europe NV Leuven, Belgium), were placed subcutaneously on the back to triangulate the heart. The leads were fastened with sutures and the snakes were given an intraperitoneal injection of the antibiotic enrofloxacin (Baytril®; 2–3 mg kg^{-1}) to prevent infection. The surgery normally lasted 30 min and the snakes spontaneously resumed breathing within 15 min and appeared to exhibit normal behaviour on the following day. In four other snakes, the heart was burned in several places avoiding any occlusion of coronary arteries, as a sham operation.

Measurement of blood pressure, heart rate and electrocardiograms

Arterial blood pressures were measured by connecting the catheters to disposable pressure transducers (Baxter Edward model PX600, Irvine, CA, USA) positioned at heart level, and heart rate (f_H) was derived from the pulsatile arterial pressure. The signal from the pressure transducers was amplified by an in-house-built preamplifier and calibrated daily against a static water column. The ECG signal was amplified 200 times using an in-house-build preamplifier and filtered with a low band/high band pass filter (0.4 Hz and 40 Hz, respectively). All signals were collected at 100 Hz using a BioPac MP100 data acquisition system (BioPac, Goleta, CA, USA) connected to a personal computer.

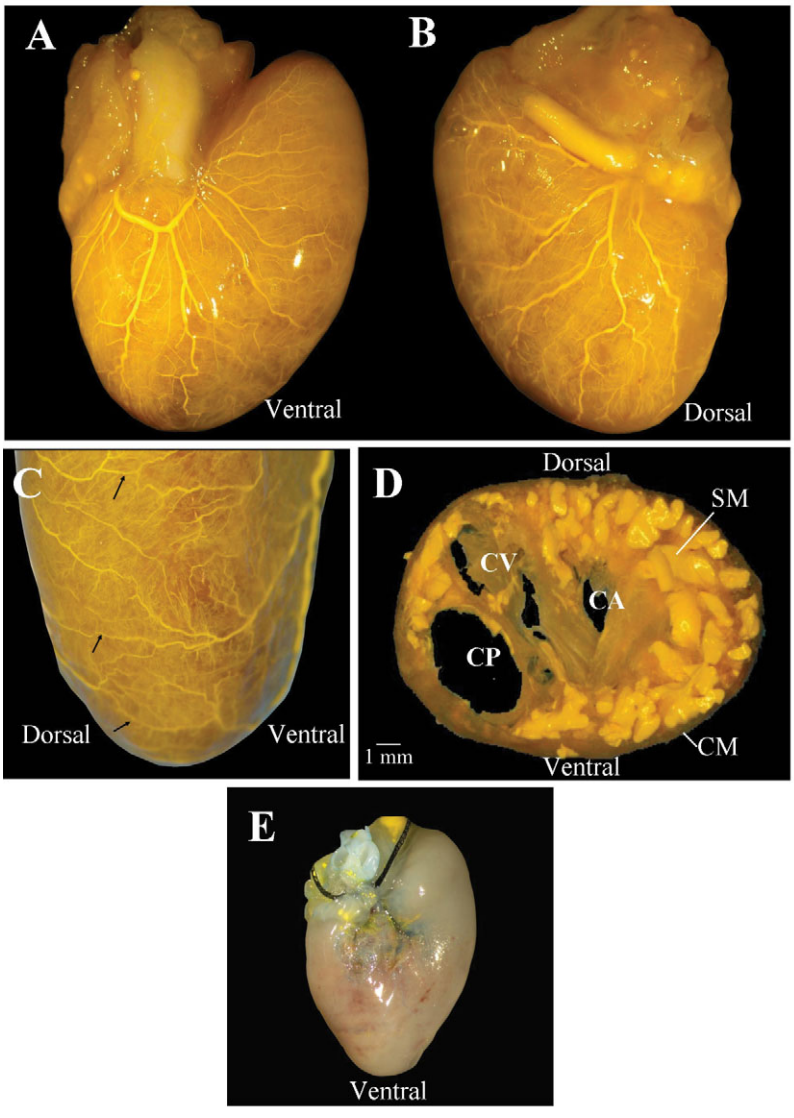

Fig. 1. The coronary arterial tree in the South American rattlesnake (*Crotalus durissus*). (A,B) Ventral and dorsal views of the ventricle where the coronary arteries have been filled with Microfil. (C) Collateral vessels of the coronary arteries. The black arrows indicate multiple epicardial anastomoses that connect coronary arteries between adjacent perfusion beds. (D) The spongy myocardium (yellow) and the thin outer compact layer (note that Microfil was injected directly into the ventricle cavity). CA, cavum arteriosum; CM, compact myocardium; CP, cavum pulmonale; CV, cavum venosum; SM, spongy myocardium. (E) Ventral view of a heart from a rattlesnake with occluded arteries. Microfil was injected in the right systemic arch to confirm successful occlusion of the coronary arteries.

Experimental protocol

The experiments were designed to investigate whether occlusion of the coronary arteries reduced maximal cardiac performance. The snakes were studied over 4 days after operation and were kept in plastic boxes maintained at 30°C within a climatic chamber (Fanem, SP, Brazil). At days 1 and 4, ECG, blood pressure and heart rate were measured. During measurements the catheter and the ECG leads were passed out of the climatic chamber to minimise disturbances, and measurements were taken no less than 90 min after connecting the catheters. Measurements were performed at rest after an undisturbed period of approximately 90 min and during 5 min of enforced activity where the snakes were provoked to rattle their tail and strike repeatedly at the investigators.

Visualisation of the coronary vessels and verification of the coronary ablation

Upon termination of the physiological measurements, all snakes were sacrificed by injection of the barbiturate mebumal (50 mg ml^{-1}) and the hearts were removed and arrested in diastole by transfer to phosphate buffer for 15 min. The coronary network was cleared of blood during this period. To evaluate whether the coronary arteries were indeed ablated, the right systemic arch was cannulated with a metal cannula and coloured silicone rubber (Microfil MV-122; Flow Tech, Carver, MA, USA) was infused at a pressure of 6 kPa over 12 min. Infusion of Microfil in non-occluded control hearts served to visualise the coronary vasculature. The hearts were then immersed in a 4% formaldehyde solution for 48 h and stored in phosphate buffer at 4°C.

Data analysis and statistics

The data were analysed using Acknowledge (3.0) data analysis software (Biopac Systems, Inc., CA, USA). All values are means ± s.e.m. Differences considered statistically significant from control values at a 95% level of confidence (P<0.05) were evaluated using paired and unpaired t-tests.

RESULTS

Visualisation of coronary arteries and collaterals

Fig. 1A,B shows the heart from a control snake in which the coronary arteries were filled with Microfil. The main coronary artery descends along the right wall of the right aortic arch and bifurcates into a dorsal and a ventral artery. The trunk of the coronary artery itself does not reach the apex of the heart, but gives off collaterals on both sides that extend over the entire ventricle. Numerous collateral arteries are distributed across the entire ventricle and form numerous anastomoses (Fig. 1C) connecting epicardial coronary arteries between adjacent perfusion beds as a fine, extensive network. Fig. 1D illustrates two distinct layers of the myocardium in a cross section of a heart filled with Microfil through the ventricular cavity. The inner spongy myocardial layer is more than 10 times thicker than the outer compact myocardial layer.

Injection of Microfil in the occluded hearts confirmed that occlusion of the coronary arteries was successful in all snakes, as shown in the example from one snake in Fig. 1E.

Haemodynamic variables

Mean systemic blood pressures (P_{sys}) and heart rates (f_H) at rest and during enforced activity at 1 and 4 days after surgery are presented in Fig. 2. Most snakes exhibited minor periodic increases in heart rate, probably associated with ventilation, and mean values were taken for non-ventilatory periods. Both at days 1 and 4, enforced activity caused an immediate rise in P_{sys} of both sham-operated snakes and snakes with coronary occlusion. Occlusion of the vessels had no significant effect on P_{sys} during enforced activity, although P_{sys} tended to be higher in the snakes with coronary occlusion. Both at rest and during enforced activity at day 4, there was a significant reduction in P_{sys} compared with day 1.

f_H of both sham-operated snakes and snakes with coronary occlusion also increased significantly during enforced activity at days 1 and 4. In general, occlusion of the coronary circulation did not elicit any significant differences in f_H of the two groups after 1 or 4 days. However, at rest at day 4 there was a significant difference between the two groups.

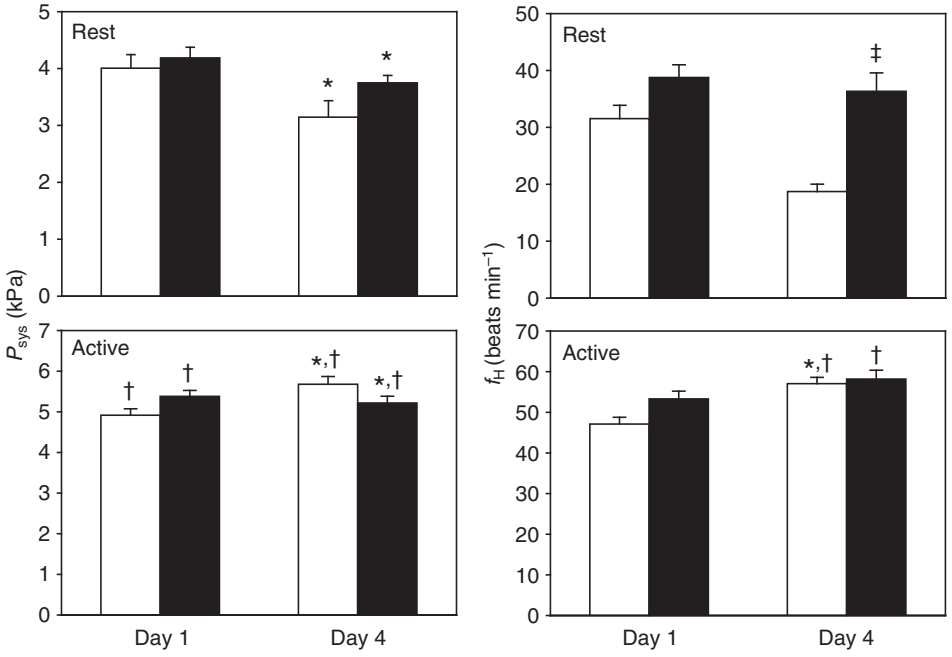

Fig. 2. Blood pressure (P_{sys}) and heart rate (f_H) in active and resting rattlesnakes, *Crotalus durissus*, at 1 day or 4 days after occlusion of the coronary arteries. Black bars represent snakes with occluded coronary arteries. White bars represent sham-operated snakes. Values are means ± s.e.m. Control snakes (N=4); snakes with coronary occlusion (N=6). *Significant difference from day 1; †significant difference from rest; ‡significant difference from sham-operated snakes.

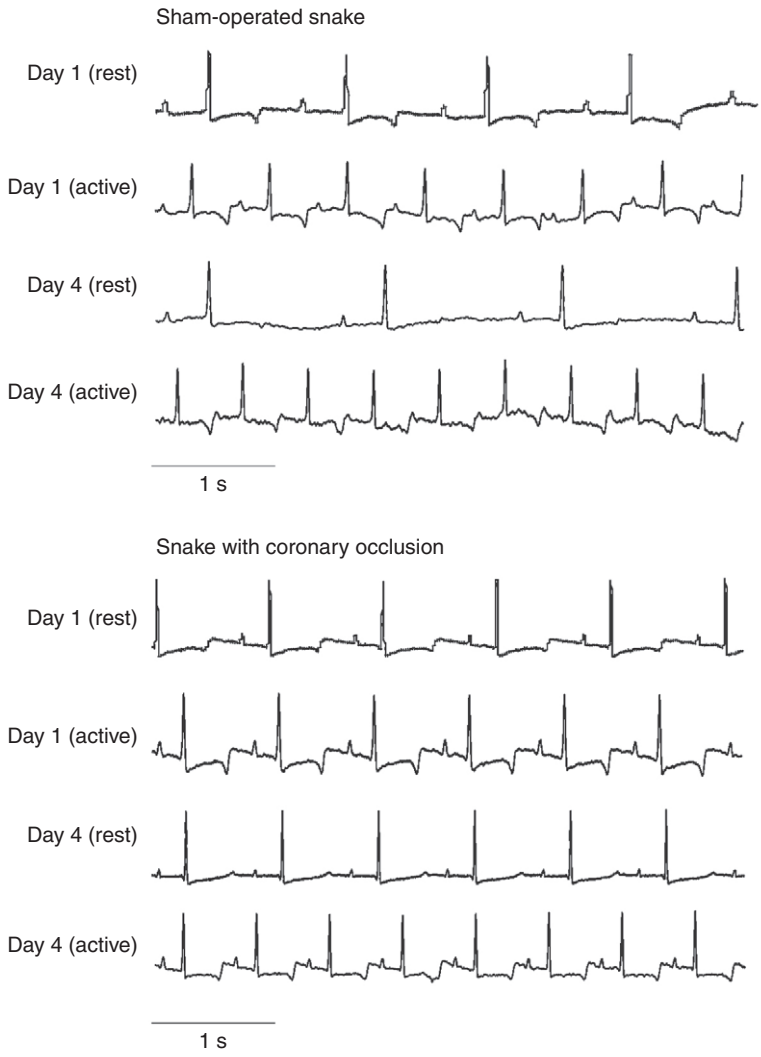

Fig. 3. Examples of electrocardiograms (ECGs) from rattlesnakes (*Crotalus durissus*) after occlusion of the coronary circulation and from sham-operated snakes (see text for further explanation).

Electrocardiogram

Electrocardiograms (ECGs) of the snakes resembled those of other vertebrates, with a small upright P wave followed by a large QRS complex, small T wave, and a short TP interval (Fig. 3), but there were no signs of cardiac abnormalities upon occlusion of the coronary arteries (see Fig. 3 for examples of ECGs). In general, the T wave was reversed in sham-operated snakes and those with coronary occlusion, and there were no detectable differences in the ECG between the two groups. The QT interval, taken as the period from the beginning of the QRS complex to the end of the T wave (Fig. 4) reflects the duration of the ventricular action potential, including repolarisation. The duration of the QT interval decreased significantly during activity in both groups as heart rate increased, but when expressed relative to the RR interval, the QT interval was prolonged during activity (Fig. 4D). Increased heart rate during activity also extended the relative duration of the QRS complex (Fig. 4A), without prolonging the ST and PR intervals (Fig. 4B,C). Overall, there were no differences in the ECG between the sham-operated snakes and the snakes with coronary occlusion except during rest on day 1.

DISCUSSION

The rattlesnake myocardium resembles that of other reptiles and is composed of a thin outer compact layer, similar in appearance to the mammalian myocardium, and a thick spongy inner myocardium composed of a network of sinusoids and large channels that emanate from the ventricular chamber (Grant and Regnier, 1926; Erhart, 1935; Mackinnon and Heatwole, 1981; Farrell et al., 1998). The Microfil injections revealed an extensive epicardial coronary arterial network with multiple anastomoses connecting epicardial coronary arteries from adjacent perfusion beds. It is, however, difficult to establish whether the coronary circulation of *Crotalus* is more extensive than other reptiles because previous studies on reptiles only described the major vessels (see Farrell et al., 1998). Clearly it would be of interest to use Microfil for detailed descriptions of the coronary arteries in other reptiles. In spite of the extensive coronary circulation to the outer myocardium, our study shows that ablation of the coronary blood supply does not affect the ability to maintain blood pressure and heart rate at rest and during short-term activity or cause abnormalities in the electrocardiogram. We also did not observe any indication of necrosis in the hearts with ablated coronary blood supply (data not shown).

Heart rate and blood pressures were similar to previous reports on cannulated awake rattlesnakes (Skals et al., 2005), but f_H was higher than in rattlesnakes that had recovered longer from a less invasive procedure (Campbell et al., 2006). The tachycardia 4 days after surgery in snakes with occluded coronaries is probably due to the more invasive treatment, but shows that *Crotalus* can maintain

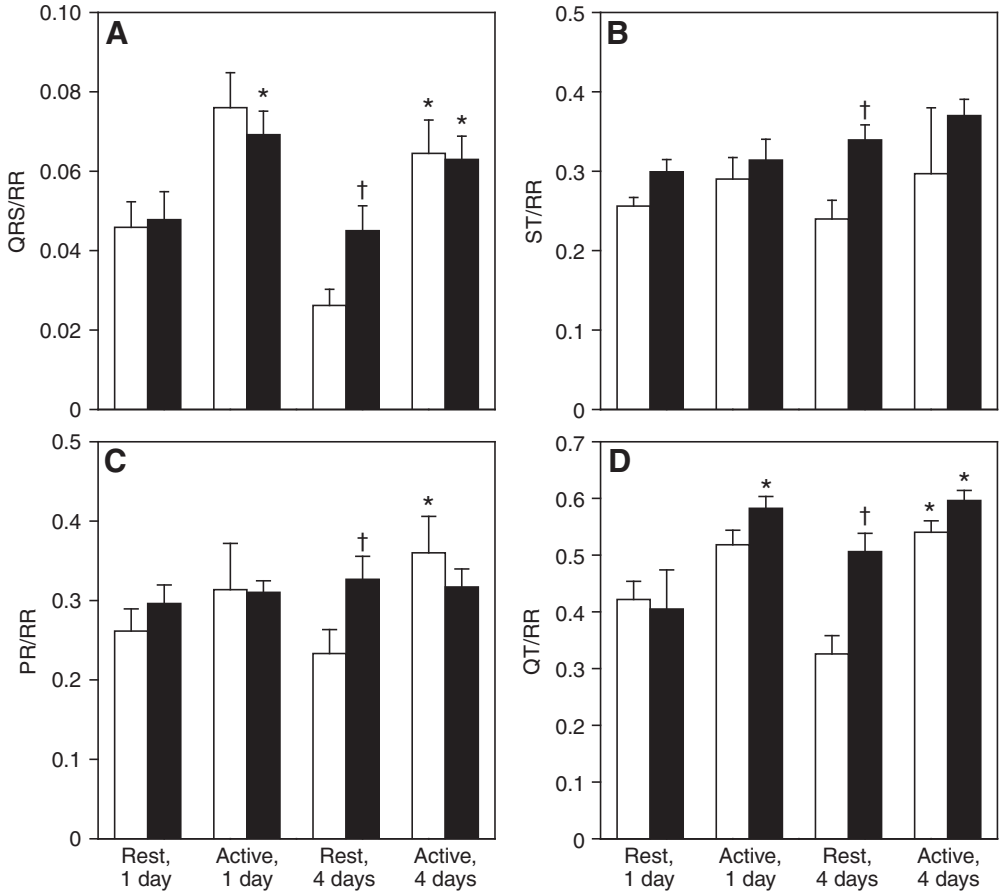

Fig. 4. The duration of the QRS interval (A), ST interval (B), PR interval (C) and QT interval related to heart rate (RR) of the ECG waveform for sham-operated rattlesnakes (*Crotalus durissus*; white bars) and snakes with occluded coronary circulation (black bars) after 1 day and 4 days. Values are means ± s.e.m. Control snakes (N=4); snakes with coronary occlusion (N=6). *Significant difference from rest; †significant difference from sham-operated snakes.

high blood pressure and heart rate without a coronary blood supply. As in other snakes, P_{sys} and f_H increased immediately during activity, which is likely to stem from increased sympathetic tone (Stinner and Ely, 1993; Wang et al., 2001). The cardiovascular response to enforced activity was similar to previous measurements on rattlesnakes, and heart rate attained maximal values for that temperature (T.W. and A.S.A., unpublished observations). Occlusion of the coronary arteries did not influence the rise in P_{sys} and f_H during enforced activity and shows that rattlesnakes can increase cardiac work substantially during short bouts of intensive exercise without coronary perfusion. This may imply that the cardiomyocytes in the outer compact myocardium are either very hypoxia-tolerant or that they derive oxygen from the lumen. Given that the compact myocardium seems surprisingly thin, it is possible that diffusion distance is sufficiently short to maintain oxygen delivery. However, venous P_{O_2} is probably very low during exercise, which would reduce the P_{O_2} gradient driving diffusion of oxygen from the lumen to the outer myocardium. It is not known how much the compact layer contributes to force development during each heart beat, so it is also possible that its major function is to provide a surrounding shell to prevent cardiac distension. Nevertheless, given the extensive network of the coronary arteries, it seems that the compact layer receives a large supply of oxygen, which would indicate a high oxygen requirement of that tissue. Electrically paced ventricular strips from the European viper, which hibernates at low temperature, shows a pronounced anoxia tolerance (Poupa et al., 1978), whereas contractility of heart strips from pythons is markedly reduced in hypoxia (Zaar et al., 2007). The hypoxia sensitivity of the cardiac muscle of rattlesnakes remains to be studied, but given the high temperature in our studies, it seems very unlikely that these hearts would be able to continue force production for 4 days without oxygen. The surprising thing is how much blood supply it appears to receive.

Although our study shows that rattlesnakes with ablated coronary arteries can sustain normal cardiovascular response to short bouts of exercise, our study does not rule out the possibility that the coronary arteries may be important for cardiac performance during longer periods with elevated metabolism. We studied the snakes at 30°C to increase metabolism, but it would be interesting to assess importance of the coronary circulation on myocardial oxygen supply during digestion where myocardial oxygen demand is high and when venous blood returning to the heart is low in oxygen (Andrade et al., 1997; Overgaard and Wang, 2002; Arvedsen et al., 2005). As in rattlesnakes, the fish heart is not solely dependent on coronary blood flow (Daxboeck, 1982; Farrell and Steffensen, 1987; Farrell, 2002), but the coronary circulation becomes essential during hypoxia and intense swimming in rainbow trout (Steffensen and Farrell, 1998). As a notable exception, skipjack tuna, where 60% of the heart consists of compact muscle seems to depend on coronary circulation (Farrell et al., 1992).

In turtles and alligators, the compact myocardium also receives some blood supply directly from the ventricular lumen and this transmyocardial blood supply may account for as much as 30% of the coronary perfusion (Brady and Dubkin, 1964; Voboril and Schiebler, 1970; Kohmoto et al., 1997). Whether a similar contribution is present in the rattlesnake remains uncertain. It is possible that there are channels between the spongy and compact myocardium, as in alligators (Kohmoto et al., 1997) that delivers

sufficient oxygen to the heart. Turtles have a large left–right shunt during exercise (Shelton and Burggren, 1976; West et al., 1992; Krosniunas and Hicks, 2003) and it was suggested that the shunting of oxygen-rich blood throughout the ventricle serves to oxygenate the myocardium (Farmer, 1999; Farmer and Hicks, 2002). It is not known whether *Crotalus* exhibit a similar shunt pattern during exercise, but adrenergic stimulation can certainly cause a large left–right shunt (Galli et al., 2007).

The unaffected f_H and P_{sys} were consistent with a lack effects on the ECG. Electrical anomalies and arrhythmia have been reported during myocardial hypoxia in the turtle *Trachemys scripta* (Farmer and Hicks, 2002), where hypoxia increased the Q wave, elevated the ST segment and lengthening the P–R interval (Farmer and Hicks, 2002). These changes are similar to those observed in mammals, in which an inverted T-wave is a reliable indicator for cardiac ischemia (Kern, 2005). Although a similar interpretation was proposed for turtles (Farmer and Hicks, 2002), this does not seem to be the case for the rattlesnakes because inverted T waves were observed equally often in sham-operated snakes (Fig. 3). Similarly, the T-wave is often inverted in toads and lizards (Zaar et al., 2004; Liu and Li, 2005), and may be explained by anatomical differences affection the direction of the ventricular repolarisation. In humans, transmural infarct of the left ventricle elevates the ST interval, whereas a depression of the ST interval results from subendocardial infarction. Occlusion of the coronary circulation did not affect the ST interval in rattlesnakes.

In conclusion, Microfil injection revealed an extensive epicardial coronary arterial network in *Crotalus*, and the snakes were able to maintain normal blood pressures and heart rate at rest as well as the ability to increase blood pressure and heart rate during short-term activity for at least 4 days after occlusion of the coronary arteries.

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