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THE ROLE OF THE PERICARDIUM AND THE EFFECTS OF ADRENALINE AND CHANGES IN OXYGEN TENSION ON THE PERFORMANCE OF AN *IN SITU* PERFUSED CROCODILE HEART

MICHAEL AXELSSON[1] AND CRAIG E. FRANKLIN[2]

[1]*Department of Zoophysiology, University of Göteborg, S-413 90 Göteborg, Sweden* and [2]*Department of Zoology, University of Queensland, Brisbane 4072, Australia*

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Summary

An *in situ* perfused crocodile (*Crocodylus porosus*) heart preparation was used to examine the mechanical responses of the heart to increases in adrenaline concentration, to a decrease in oxygen tension and to opening of the pericardium. Starling and power curves were constructed before and after these experimental manipulations. Increasing adrenaline concentration in the perfusate from 5 nmol l^{-1} to 0.5 μmol l^{-1} produced a significant increase in heart rate and a decrease in stroke volume, leaving cardiac output unchanged. With maximal adrenergic stimulation, the left ventricle was able to generate greater power outputs at high right aortic output pressures; however, the right ventricle showed a decrease in performance with increasing output pressure.

Decreasing the P_{O_2} of the perfusate to 10 kPa resulted in a significant bradycardia. Both the flow and pressure-generating capabilities of the perfused heart preparation were reduced, although the heart was able to maintain low work levels at this P_{O_2}.

Opening the pericardium permitted greater movement/expansion of the cardiac chambers and resulted in an increase in heart rate. Higher flows were generated at low filling pressures during the input pressure challenge as a result of an increase in the sensitivity of the Starling response.

Key words: *Crocodylus porosus*, pressure, flow, cardiac output, heart rate, stroke volume, bradycardia, catecholamines.

Introduction

Recently, an *in situ* perfused crocodilian heart preparation (Franklin and Axelsson, 1994) was developed, enabling the intrinsic mechanical properties of the heart to be examined. This is a double-perfused heart preparation with all three outflow tracts (pulmonary, right and left aortic arches) cannulated and their respective flows and pressures monitored. The perfused *Crocodylus porosus* heart was able to generate physiological levels of flow and power, an important consideration if the results are to be confidently extrapolated to the situation *in vivo*. Previous *in vivo* studies on crocodilians have concentrated on the timing of the phasic relationships between pressure and flow in the ventricles and outflow tracts of the heart. Of particular interest was the elucidation of the conditions that cause a shunting of blood away from the pulmonary outflow tract (PA) and into the left aorta (LAo), the right-to-left shunt (Grigg and Johansen, 1987; Axelsson *et al.* 1989, 1991; Shelton and Jones, 1991; Jones and Shelton, 1993).

The aim of this study was to focus on the intrinsic responses of the crocodilian heart to changes in adrenaline concentration and to a decrease in oxygen tension as well as to determine the role of the pericardium in cardiac function. This type of fundamental cardiac information has not been measured in crocodilians, and the perfused heart preparation lends itself to this type of investigation. Perfused turtle heart preparations have been useful in investigating the effects of factors such as hypoxia, temperature and catecholamines on cardiac dynamics (Wasser *et al.* 1990; Jackson *et al.* 1991; Comeau and Hicks, 1994; Farrell *et al.* 1994; Franklin, 1994; Hicks and Comeau, 1994). Furthermore, a wealth of information has been obtained from the *in situ* perfused fish heart preparation in which responses to a wide variety of stimuli and conditions have been documented (Farrell *et al.* 1983, 1986; Franklin and Davie, 1991, 1992, 1993).

Materials and methods

Animals

Experiments on the estuarine crocodile *Crocodylus porosus* Schneider (body mass 1.16±0.06 kg; mean ± S.E.M., N=11) were performed in the Department of Zoology at the University of Queensland. The crocodiles were obtained from a commercial farm at Edward River, Cape York, and transported to the University of Queensland where they were held outdoors

in a 4 m diameter fibreglass tank partly filled with fresh water heated to 30 °C. They were fed fish and meat scraps and had access to a platform on which they could bask.

Perfused heart preparation

The preparation used is the same as that described by Franklin and Axelsson (1994). Briefly, the crocodile was killed by a single sharp blow to the head (an approved method suitable only for small animals (University of Queensland AEEC, ZOO/331/91/NSRG), weighed and then immediately ventilated with O_2. The heart and surrounding vessels were exposed and the blood system heparinised. Stainless-steel cannulae were inserted into the left and right pulmonary veins and the hepatic vein. These provided inputs for the Ringer for the left and right sides of the heart. Output cannulae were put into the right aorta (RAo), left aorta (LAo) and pulmonary artery (PA). The remaining systemic veins and arteries were tied off. The heart, perfused *in situ*, was placed into a saline bath and the input cannulae were connected to constant pressure devices. The output cannulae were connected to separate pressure heads which could be adjusted independently.

The Ringer (see Franklin and Axelsson, 1994, for its composition), contained 5 nmol l^{-1} adrenaline and was gassed with 97 % O_2 and 3 % CO_2 to give a pH of 7.4–7.5 at 23 °C.

Instrumentation

Pressures were measured at the tips of the stainless-steel cannulae (see Franklin and Axelsson, 1994) using Statham P23XL pressure transducers. Flows in the RAo, LAo and PA were measured using a Doppler system (University of Iowa, Bioengineering, model 545C-4) with extracorporeal flow probes (Titronics Medical Instruments) incorporated into the outflow tubing. The pressure and flow signals were amplified and displayed on Grass Polygraphs (model 79D). Heart rate was determined from the RAo flow signals using a Grass 7P44 tachograph module. Pressures, flows and heart rate signals were also directed to a computer, digitally converted and then analysed by AD/DATA (P. Thorén, Astra AB, Sweden).

Experimental protocols

Control conditions and cardiac challenge tests

By adjusting the left and right atrial filling pressures (0.15–0.25 kPa), the heart preparation was set to deliver a total cardiac output of approximately 40 ml min^{-1} kg^{-1} body mass (i.e. left and right ventricular outputs of 20 ml min^{-1} kg^{-1} body mass) against output pressures of 3.5 kPa in the RAo and LAo and 2.0 kPa in the PA. The heart rate was determined by the intrinsic properties of the pacemaker cells. The heart was allowed to stabilise to these conditions before the performance was assessed by filling pressure and output pressure challenges.

The effect of filling pressure on cardiac output (Starling response) was tested first. Pressures to the left and right atria were simultaneously increased in 0.1 kPa steps from approximately 0 kPa to a maximum of 0.8 kPa. Output pressures were kept constant during the increases in filling pressure. The left and right ventricular outputs were determined.

The heart was returned to control conditions and allowed to stabilise before the output pressure challenge test. The left ventricle was tested by increasing the output pressure in the RAo in steps of 0.5–1.0 kPa up to a maximum of 10.5 kPa. Pressure in the LAo was simultaneously increased and matched the RAo pressure to prevent the perfusate from being directed through the foramen of Panizza. The right ventricle was tested in a similar way up to a maximum of 8.5 kPa; to prevent the perfusate from being shunted into the LAo, the LAo and RAo output pressure were kept above the PA pressure during this test.

The above cardiac challenge tests were repeated after each of the following experimental treatments.

Effect of adrenaline

The response of the perfused heart preparation to a cumulative increase in adrenaline concentration was examined. Adrenaline was added to the Ringer in the reservoirs to give concentrations of 5 nmol l^{-1}, 50 nmol l^{-1}, 0.5 μmol l^{-1} and finally 5 μmol l^{-1}. The increasing doses of adrenaline were added to the preparation while it was working under the initial control conditions. At each adrenaline concentration, the heart was allowed to equilibrate before adrenaline concentration was increased further. The filling and output pressure challenge tests were not carried out until the maximum adrenaline concentration of 5 μmol l^{-1} had been reached.

Effect of lowering the P_{O_2} *of the perfusate*

The effects of a decrease in perfusate P_{O_2} were examined. The control perfusate was gassed with 97 % O_2 and 3 % CO_2, which gave P_{O_2} values of over 93 kPa. The P_{O_2} of the perfusate was reduced by bubbling with a gas mixture containing 87 % N_2, 10 % O_2 and 3 % CO_2. The P_{O_2} of the perfusate was continuously monitored with a Radiometer O_2 electrode and meter (PHM73). Once the P_{O_2} had reached 10–11 kPa (between 15 and 20 min), the performance of the heart was tested using the flow and output pressure challenges.

Effect of opening the pericardium

The role of the pericardium was investigated by carefully opening it on the ventral surface with a cross-shaped cut that exposed the ventricle and both atria. The performance of the exposed heart, determined from the filling and output pressure challenge tests, was compared with the performance of the heart working in the presence of 5 μmol l^{-1} adrenaline.

Data analysis

From the pressure, flow and heart rate (f_H) data stored on the computer, cardiac outputs, stroke volume (V_S) and power output were calculated. Left ventricular output = RAo flow (F_{RAo}), and left ventricular stroke volume (V_S) = F_{RAo}/f_H. Right ventricular output = F_{PA} + F_{LAo}, and right ventricular V_S=(F_{PA}+F_{LAo})/f_H. Flow, cardiac output and V_S were

normalised per kilogram body mass. Power output (in mW) was calculated for each ventricle using the following equations:

Left ventricular power output =

$$0.0167[(P_{RAo} - P_{LungV}) \times F_{RAo}], \quad (1)$$

Right ventricular power output =

$$0.0167[(P_{PA} - P_{VCava}) \times F_{PA}] + 0.0167[(P_{LAo} - P_{LungV}) \times F_{LAo}], \quad (2)$$

where, P_{RAo}, P_{LungV}, P_{PA}, P_{VCava} and P_{LAo} are pressures (kPa) in the right aorta, lung veins, pulmonary artery, vena cava and left aorta, respectively; F_{RAo}, F_{PA} and F_{LAo} are flows (ml min^{-1}) in the right aorta, pulmonary artery and left aorta, respectively, and 0.0167 is the conversion factor to calculate power in milliwatts.

A detailed description of the calculations used is given by Franklin and Axelsson (1994). The results are presented as means ± S.E.M. Statistical differences (P<0.05) were determined using a randomisation test for matched pairs followed by a modified Bonferoni compensation for repeated testing (Holm, 1979). In order to avoid repeated testing, we selected three points where we tested for significant differences between control and treatment (adrenaline, P_{O_2} and pericardium): one point at a low filling or output pressure, one at a mid-point in the range and one close to the maximal input/output pressure tested. The other points were not tested for significant differences. For the Starling curves, the 0.1, 0.4 and 0.75 kPa values were used for testing both the right and the left ventricle. For the power curves, the 3.5/2.0, 6.5/5.0 and 10.0/8.0 values were used for both the right and left ventricle.

Results

Effect of adrenaline

With the heart working under control conditions, increasing the concentration of adrenaline in the perfusate resulted in a significant elevation in heart rate (Fig. 1A). A maximum heart rate of 39.9±1.0 beats min^{-1} was recorded at 0.5 μmol l^{-1} adrenaline, which represented a 19.1±4.6 % (5.8±1.3 beats min^{-1}) increase from heart rate at the initial concentration of 5 nmol l^{-1}. No further increase in heart rate occurred when the adrenaline concentration was elevated from 0.5 to 5 μmol l^{-1} (Table 1). Left ventricular stroke volume decreased significantly as the adrenaline concentration was increased (Fig. 1B). As a result of an increase in heart rate and a decrease in stroke volume, the left and right ventricular outputs were not affected by the increase in adrenaline concentration, nor were the left and right ventricular power outputs (Fig. 1C,D).

Table 1. *A summary of the effects of the various treatments on heart rate*

	Change in heart rate	
	(%)	(beats min^{-1})
Adrenaline (5 μmol l^{-1})	+19.1±4.6	+5.8±1.3*
Decrease in P_{O_2}	–6.2±0.7	–4.4±0.2*
Open pericardium	+8.1±1.9	+2.8±0.6*

Values are mean ± S.E.M. (N=6); asterisks indicate a statistically significant change from control values (P<0.05).

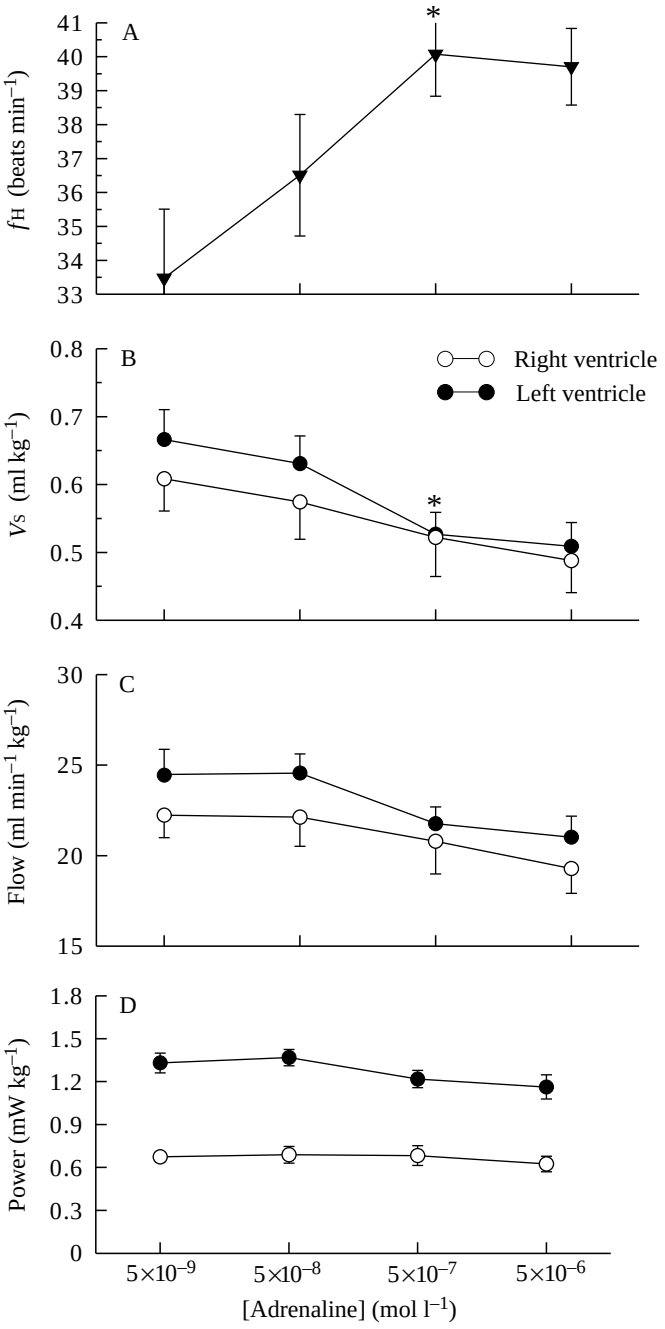

Fig. 1. Effect of logarithmic increases in adrenaline concentration in the perfusate on heart rate (f_H), left and right ventricular stroke volumes (V_S), outputs (flows) and power outputs in the perfused crocodile heart. Filled circles represent cardiac parameters for the left ventricle and open circles are cardiac parameters for the right ventricle. Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between control (5×10^{-9} mol l^{-1}) and 5×10^{-7} mol l^{-1} adrenaline (P<0.05).

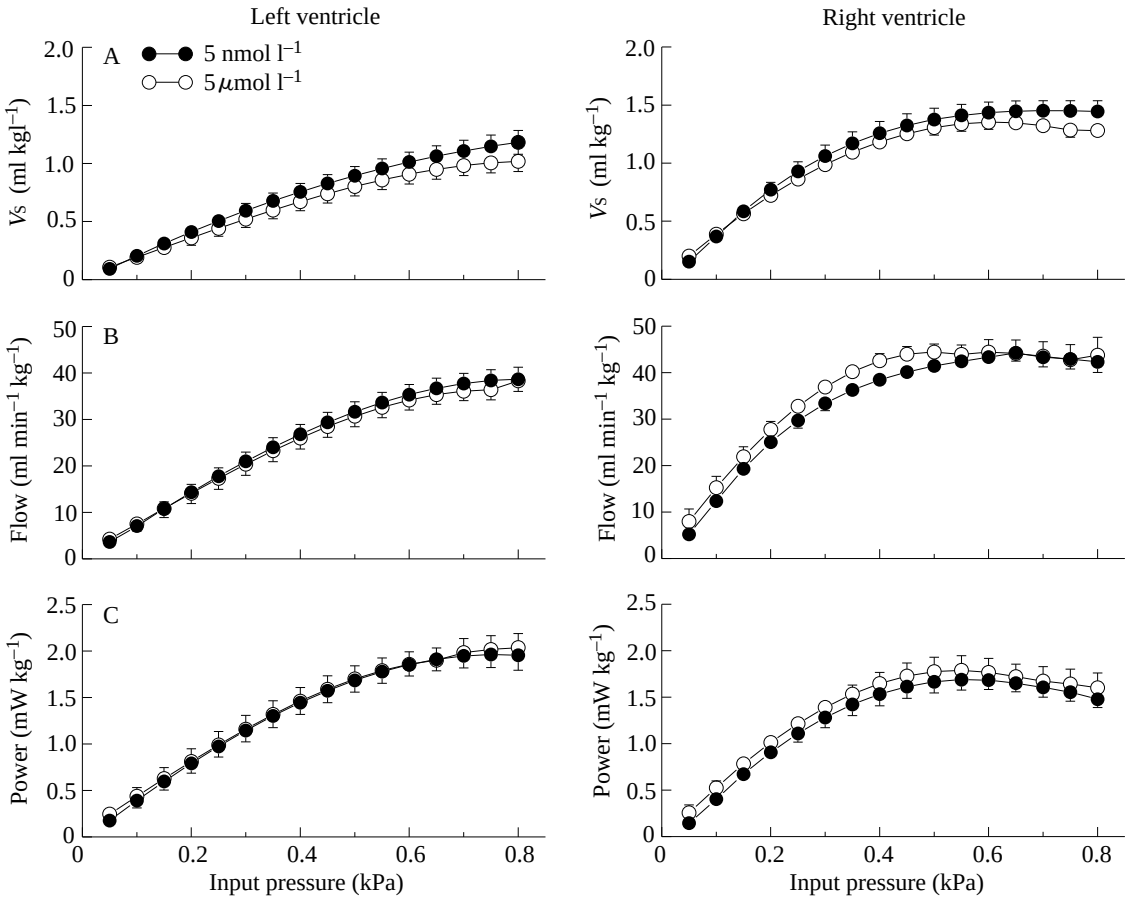

Fig. 2. Effect of increasing input pressure to the left and right side of the heart on left and right ventricular stroke volumes (*V*S) (A), flow (B) and power output (C) at 5 nmol l^{-1} (filled circles) and 5 μmol l^{-1} adrenaline (open circles). Results are means ± S.E.M. (*N*=6).

Increasing the filling pressure to the left and right atria significantly increased the respective left and right ventricular stroke volumes, ventricular outputs and power outputs in the perfused crocodile heart. There was, however, no significant difference in the responses of the heart to the filling pressure challenge at 5 nmol l^{-1} and 5 μmol l^{-1} adrenaline (Fig. 2).

Increasing left aortic output pressure from 3.5 to 10.5 kPa resulted in a significant increase in left ventricular power output. At the maximum output pressure tested (10.5 kPa), the left ventricular power output generated at 5 μmol l^{-1} adrenaline was greater than that at 5 nmol l^{-1} adrenaline (Fig. 3C). In contrast, there was a decrease in performance of the right ventricle when adrenaline concentration was increased to 5 μmol l^{-1}. The right ventricle was unable to maintain power output above approximately 6.0 kPa (Fig. 3).

Effect of lowering the P_{O_2} of the perfusate

Decreasing the P_{O_2} of the perfusate from approximately 90 to approximately 10 kPa (atmospheric P_{O_2}≈20 kPa) resulted in a significant bradycardia (see Table 1).

During the input pressure challenge to the heart, lowered P_{O_2} had no effect on left ventricular stroke volume, RAo flow or power output at low filling pressures (Fig. 4A–C). Only at higher input pressures was there a significant difference in left ventricular flow and power generation between control and reduced P_{O_2} conditions (Fig. 4). At a left atrial filling pressure of 0.8 kPa, flow and power output from the left ventricle were reduced by approximately 30 % at P_{O_2}=10 kPa. As with the left ventricle during the input pressure challenge, the lower P_{O_2} had no effect on right ventricular performance at low filling pressures. However, increasing right atrial filling pressures above 0.4 kPa resulted in reduced right ventricular stroke volumes, flows and power outputs compared with the control (Fig. 4).

The effect of the output pressure challenge during the decreased perfusate P_{O_2} is shown in Fig. 5. Left ventricular power output and flow were significantly decreased at the lower P_{O_2} only at higher output pressures. The lower P_{O_2} had a significant effect on the performance of the right ventricle during the output pressure challenge. The right ventricle failed to maintain stroke volume with increasing output pressure and this is also reflected in the decreases in right ventricular flow and power output as output pressure was increased above 4.5–5 kPa (Fig. 5).

Effect of opening the pericardium

There was a significant increase in heart rate after the pericardium was opened (see Table 1). During the input

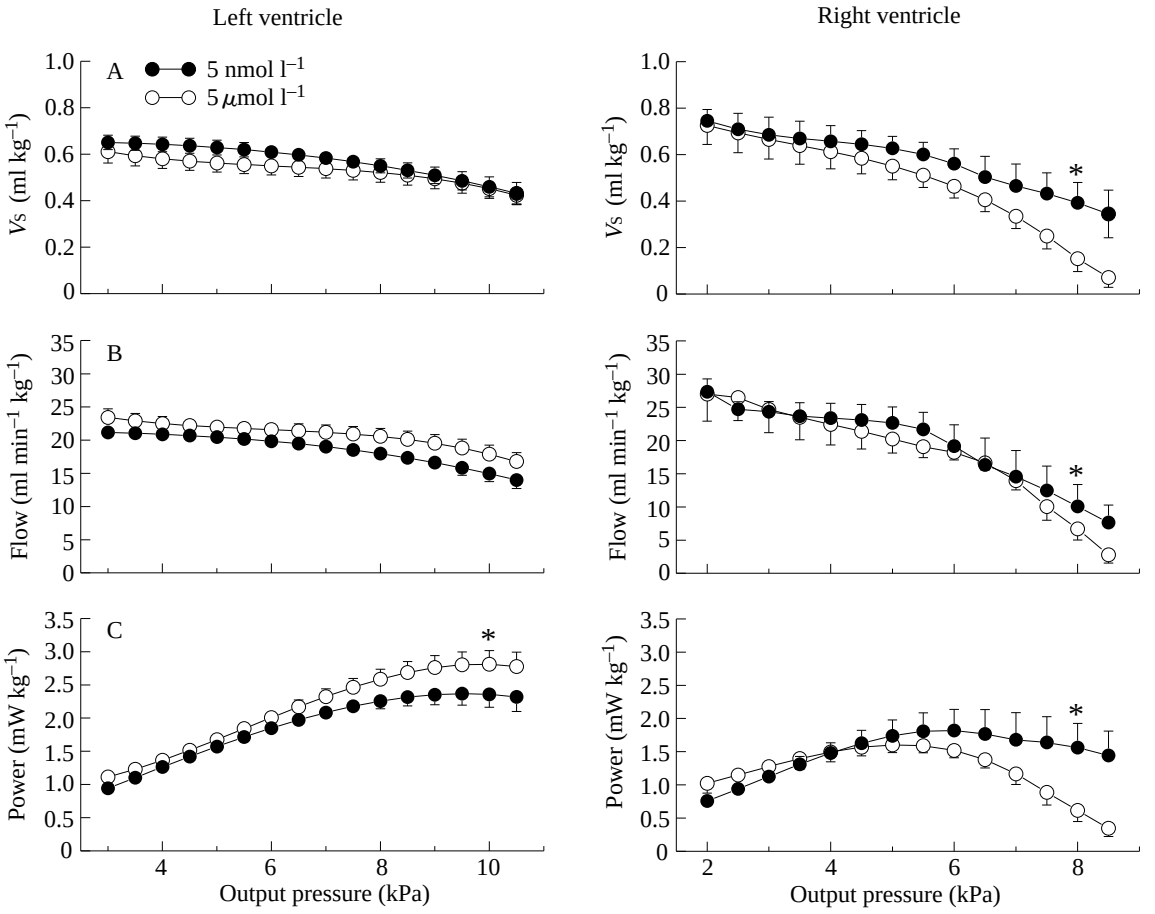

Fig. 3. Effect of increasing output pressure in the three outflow tracts (RAo, LAo and PA) on cardiac variables for the left and right ventricles at 5 nmol l^{-1} (filled circles) and 5 μmol l^{-1} adrenaline (open circles). Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between values for the two adrenaline concentrations (P<0.05). V_S, stroke volume.

pressure challenge to the heart (Fig. 6), opening the pericardium resulted in significant increases in both left and right ventricular stroke volume, flow and power output. Left ventricular stroke volume, flow and power output were all significantly elevated at 0.5 kPa. At high input pressures (>0.6 kPa), left ventricular performance was the same whether the pericardium was opened or intact. Opening the pericardium had a similar influence on right ventricle performance. At low filling pressures, there was a significant elevation of flow and power in the right ventricle. At high filling pressures (>0.6 kPa), there was no difference in right ventricular performance whether the pericardium was open or intact.

Opening the pericardium had no effect on left ventricular performance during the output pressure challenge but had a deleterious effect on right ventricular performance (Fig. 7). At high output pressures, there was a significant decrease in right ventricular stroke volume, flow and power output.

Discussion

Heart rate

The perfused *C. porosus* heart preparation remained stable throughout the experimental manipulations and maintained a regular heart beat. Heart rate was intrinsically sensitive to increases in adrenaline concentration, changes in P_{O_2} and the presence or absence of an intact pericardium. Franklin and Axelsson (1994) also found that heart rate in *C. porosus* was affected by left atrial filling pressure, with higher pressures having a positive chronotropic effect. Stretching of the left atrial wall, *via* increases in filling pressure, is known to cause the sino-atrial pacemaker to discharge at higher frequencies. Opening of the pericardium permitted greater extension of the atrial wall and this, presumably, resulted in the observed increase in heart rate.

Akers and Peiss (1963) reported a 12 % increase in heart rate in *Alligator mississippiensis* after administration of adrenaline, and Comeau and Hicks (1994) showed a 16 % increase in heart rate after adrenaline injection in an autoperfused turtle (*Pseudemys scripta*) heart preparation. This is similar to the 19 % increase in heart rate observed in the perfused *C. porosus* heart preparation when the adrenaline concentration of the perfusate was increased from 5 to 500 nmol l^{-1}. The maximum heart rate recorded from the perfused heart preparation was approximately 42 beats min^{-1}. *In vivo* heart rates in excess of 55 beats min^{-1} have been recorded from *C. porosus* at 28–30 °C and this considerably higher maximum heart rate

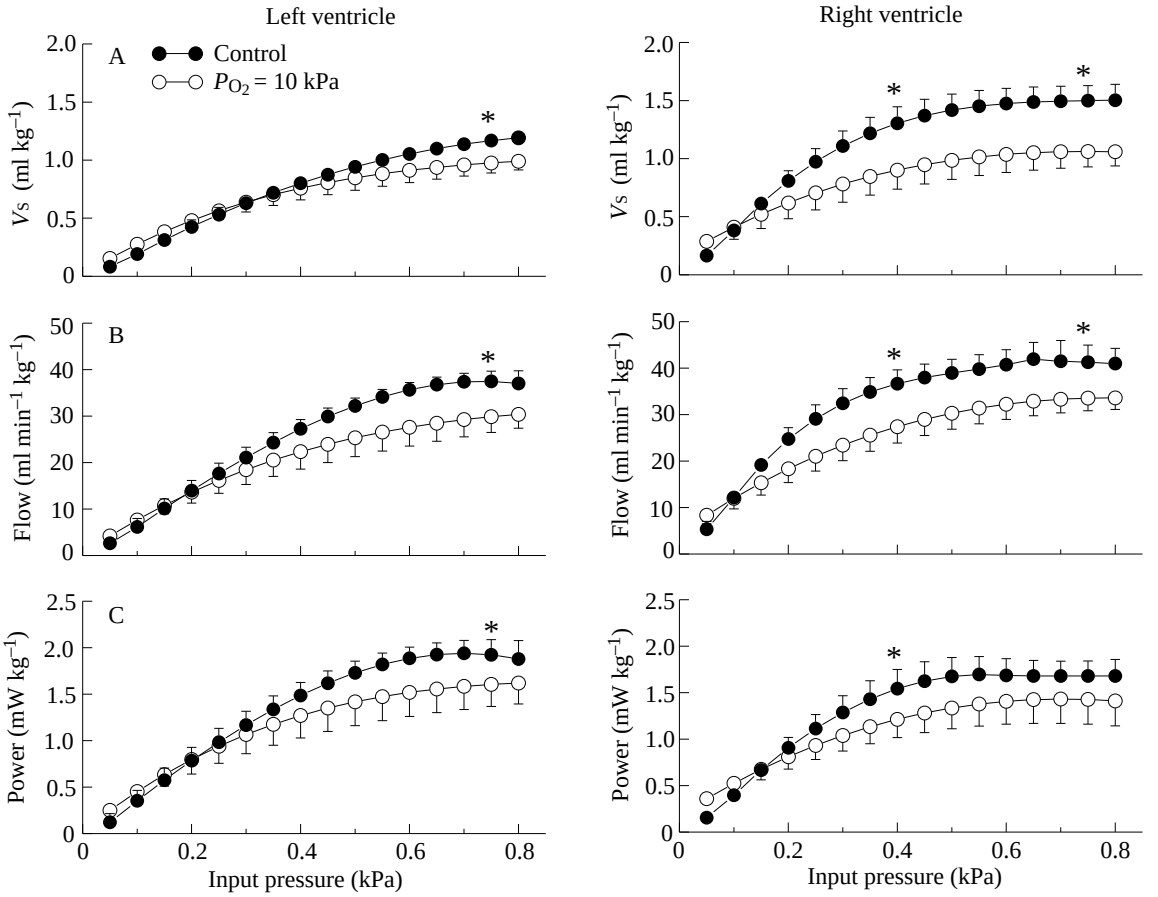

Fig. 4. Effect of reduced oxygen tension (P_{O_2}=10 kPa) on the input pressure curves for the left and right ventricles. Filled circles show the heart working under control conditions (P_{O_2}=90 kPa) and open circles the heart working at reduced oxygen tension (P_{O_2}=10 kPa). Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between values at the two oxygen tensions (P<0.05). V_S, stroke volume.

could be due to temperature effects. The perfused heart preparation was investigated at 23 °C, but the crocodiles from which the hearts were obtained were maintained at 30 °C. Even if a Q_{10} of 1.5 is assumed, this acute 7 °C decrease in temperature would account for the difference in maximum heart rates between *in vivo* and *in situ* perfused heart measurements.

It is generally accepted that increases in cardiac output in reptiles are driven by increasing heart rates (West *et al.* 1992). Increases in heart rate in excess of 100 % have been recorded from *C. porosus* (where changes from 10–15 up to 55–60 beats min^{-1} have been recorded when individuals have switched from diving to air-breathing, M. Axelsson, C. E. Franklin, S. Nilsson and G. C. Grigg, in preparation). The relatively small increase in heart rate seen with the perfused heart preparation after adrenaline administration is probably due to the higher initial heart rate and to a reduction in maximal heart rate resulting from the drop in temperature. *In vivo*, the heart of *C. porosus* is under strong inhibitory cholinergic control (M. Axelsson, C. E. Franklin, S. Nilsson and G. C. Grigg, in preparation) which produces a considerably lower heart rate compared with the aneural perfused heart preparation.

A significant bradycardia occurred when the P_{O_2} of the Ringer perfusing the heart was decreased to 10 kPa. Farrell *et al.* (1994) also found that a bradycardia occurred in the perfused turtle heart (*Chrysemys scripta*) after exposure to anoxic conditions. The intrinsic cardiac bradycardia observed in perfused *C. porosus* heart exposed to a lowered P_{O_2} is considerably smaller (5–17 %) than the bradycardia observed *in vivo* in crocodilians during fright responses and apnoea, which can be greater than 60 % (Gaunt and Gans, 1969; Smith *et al.* 1974; Wright *et al.* 1992). In the anerual perfused heart, the decrease in heart rate is a direct effect of the low P_{O_2}, while *in vivo* there is also a large vagally mediated component that affects heart rate; the combination of direct effects and neural input creates the much larger heart rate changes observed *in vivo*.

Stroke volume and cardiac output

With the perfused *C. porosus* heart working under control conditions, an increase in the concentration of adrenaline in the perfusate resulted in a decrease in left and right ventricular stroke volumes. However, as heart rate increased, left and right ventricular outputs were maintained, thus indicating a trade-off between heart rate and stroke volume. A similar finding was

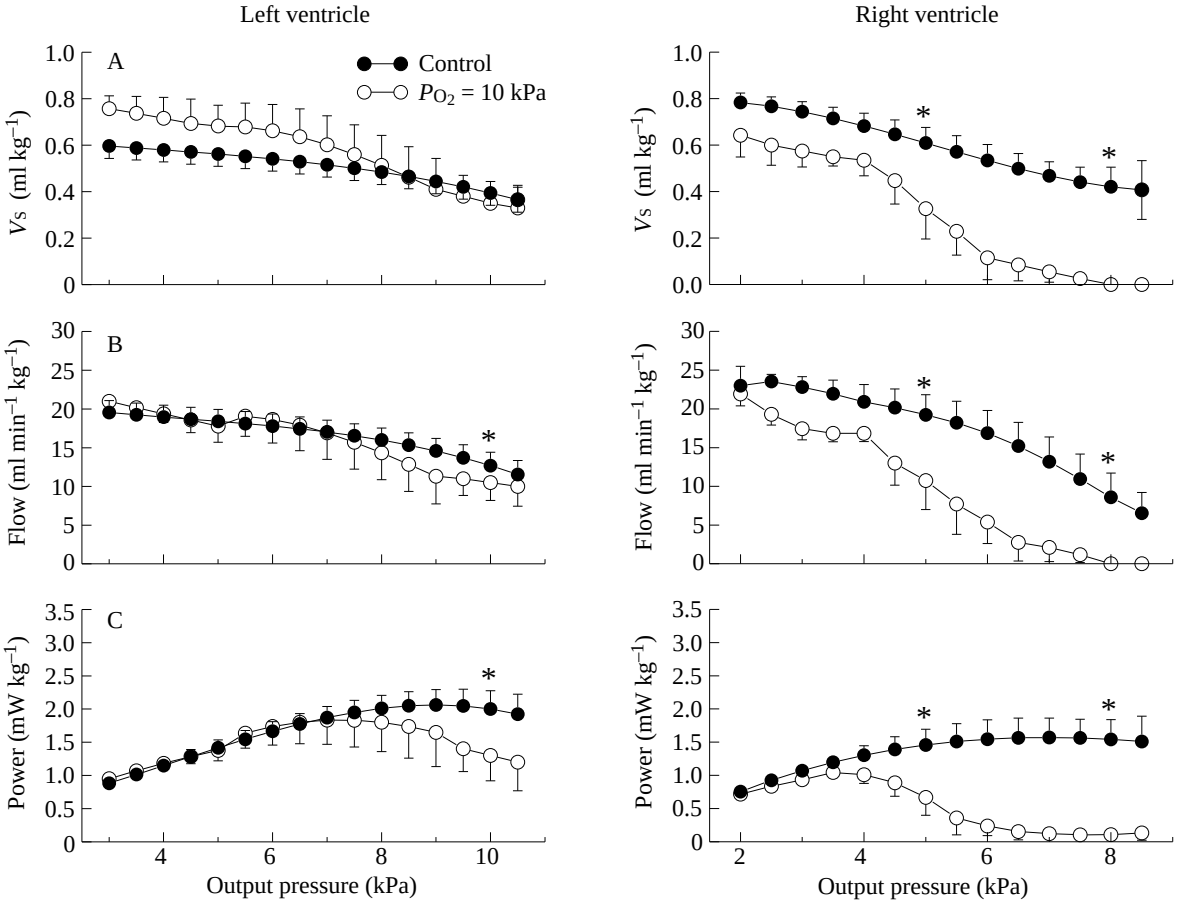

Fig. 5. Effect of reduced P_{O_2} (10 kPa) on the output pressure curves for the left and right ventricles. Open circles represent the heart working under control conditions (P_{O_2}=90 kPa) and filled circles the heart working at 10 kPa. Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between values at the two oxygen tensions (P<0.05). V_S, stroke volume.

reported by Comeau and Hicks (1994) in their paper on autoperfused turtle hearts, where an increase in heart rate was offset by a decrease in stroke volume, leaving cardiac output unchanged. At maximum adrenaline stimulation, the high heart rates appear to limit cardiac filling times. Farrell *et al.* (1983) also found, in the perfused fish heart, that the tachycardia associated with an increase in adrenergic stimulation reduced the filling time of the ventricle and compromised stroke volume because of a constant input pressure.

Increasing filling pressure to the left and right atria resulted in increases in stroke volume, ventricular output/flow and cardiac power output, demonstrating the presence of a Starling response. Increasing the adrenaline concentration in the perfusate did not affect the Starling response of the perfused *C. porosus* heart preparation. In perfused fish hearts, adrenaline increases cardiac output *via* increases in heart rate and stroke volume (Farrell *et al.* 1986; Franklin and Davie, 1992). With increases in adrenaline concentration, there is both an increase in the sensitivity of the Starling response (left shift of the curve) and an increase in the maximal flow-generating capabilities of the fish heart *via* stroke volume increases. In the perfused *C. porosus* heart, the lack of an increase in the sensitivity of the Starling response and a failure to generate greater ventricular stroke volumes with increases in adrenaline concentration may reflect the relatively small role played by stroke volume in augmenting cardiac output in crocodilians. In contrast, increases in stroke volume play a significant role in augmenting cardiac output in fish.

Decreasing the P_{O_2} of the Ringer (P_{O_2}≈10 kPa) decreased the maximum flow and stroke volume capacities of the right and left ventricles by approximately 30 %. In the perfused turtle heart, anoxia reduced maximum stroke volume by 27 % at 15 ˚C and 48 % at 5 ˚C (Farrell *et al.* 1994). In the *C. porosus* preparation, there was an apparent flattening of the Starling curves, as only at the higher filling pressures were stroke volumes affected by the decrease in P_{O_2}. Thus, at lower filling pressures (hence smaller stroke volumes and lower ventricular flows and power outputs), the perfused heart was able to tolerate the lower P_{O_2}. *In vivo*, hypoxia/apnoea in diving vertebrates generally induces a decrease in cardiac work, chiefly by initiating a bradycardia. Therefore, care must be taken when interpreting the negative effects of hypoxia observed at high work levels (high cardiac outputs or high output pressures) in the perfused crocodile heart.

Opening the pericardium affected the shape of the Starling curves generated from the left and right ventricles of *C.*

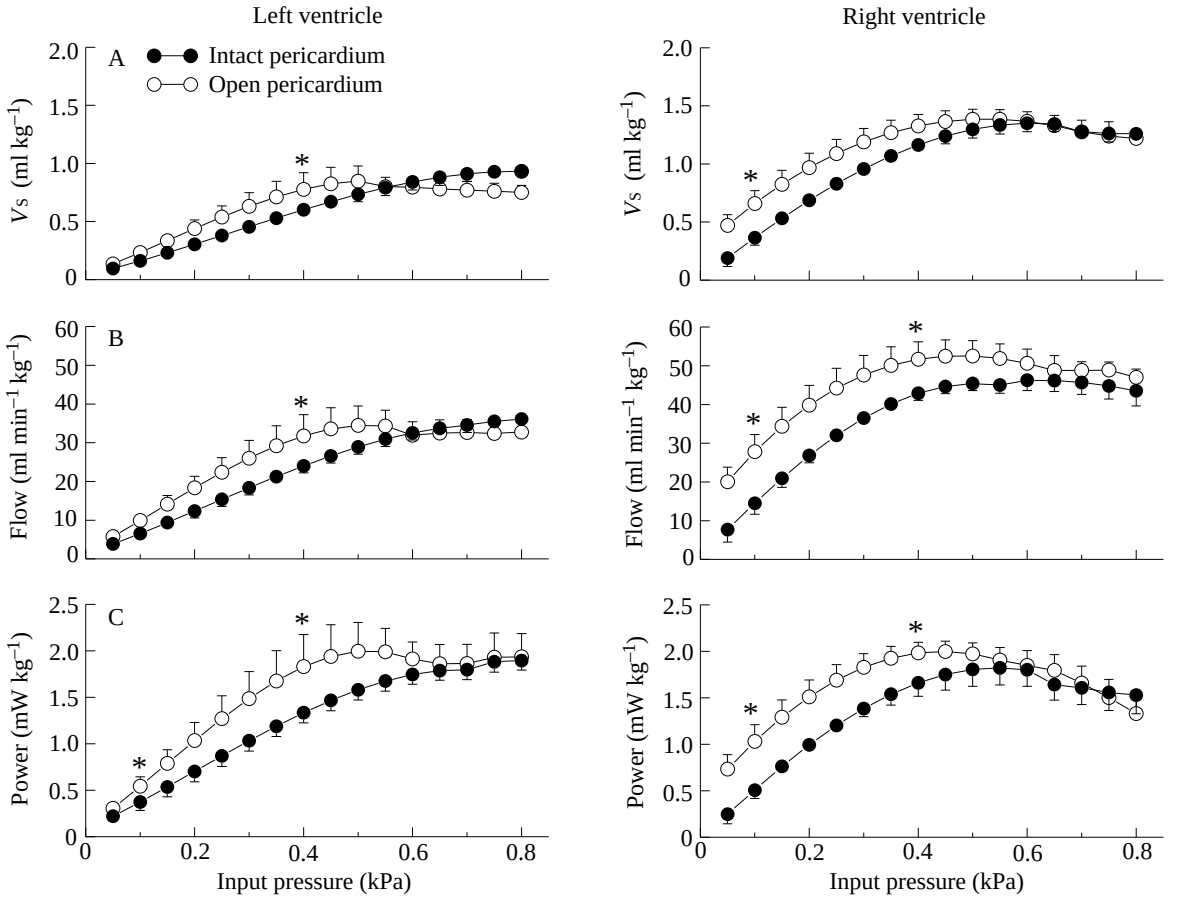

Fig. 6. Effect of opening the pericardium on the input pressure curves for the left and right ventricles. Filled circles represent the heart with an intact pericardium and the open circles the heart with the pericardium opened. Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between the two conditions (P<0.05). V_S, stroke volume.

porosus. At lower filling pressures, there was an increase in the stroke volumes generated, resulting from a shift to the left of the Starling curve (an increase in sensitivity of the Starling response). The greater expansion of the atria and ventricles at lower filling pressures after opening the pericardium would precipitate the increased sensitivity. This contrasts with the effects of opening the pericardium in trout (*Salmo gairdneri*) or dogfish (*Squalus acanthias*) (Farrell *et al.* 1988; Franklin and Davie, 1993), where there is a right displacement of the Starling curve and a reduction in stroke volume/cardiac output. *Vis-a-fronte* (negative pressure filling) filling is utilised by the trout and dogfish, and this suctional attraction phase in cardiac filling is abolished when the pericardium is opened. The crocodile heart, like the heart in mammals, birds and in other reptiles, is reliant on positive pressures to fill the atria (*vis-a-tergo* filling).

Maintenance of stroke volumes and power outputs with increasing output pressures

The ability of the crocodile heart to shunt blood away from the pulmonary outflow tract and into the left aorta can be affected by changes in both input and output pressures (Franklin and Axelsson, 1994). Shunts were not quantified or analysed in this study; instead we focused upon the overall flow and pressure capabilities of the right and left ventricles. As found by Franklin and Axelsson (1994), the left ventricle was able to maintain stroke volumes at higher output pressures than the right ventricle (i.e. the power output capability of the left ventricle was significantly greater than that of the right ventricle).

Interestingly, there was a dichotomy between the left and right ventricles in their responses to elevated adrenaline concentrations with increasing output pressure. The left ventricle showed an increase in maximum power output when adrenaline concentration was increased, whereas the power-generating capabilities of the right ventricle decreased when adrenaline concentration was increased from $5\,\text{nmol}\,l^{-1}$ to $5\,\mu\text{mol}\,l^{-1}$. An increase in adrenaline concentration typically increases the power-generating capabilities of perfused fish and turtle heart preparations (Farrell *et al.* 1986; Franklin and Davie, 1992; Comeau and Hicks, 1994) so it is unusual that high adrenaline concentrations have a negative effect on the performance of the right ventricle of the perfused crocodile heart. The presence (or lack) of adrenergic stimulation may have an effect on the shunting capabilities of the heart. How this is initiated is still unclear, but it is possible that the effect

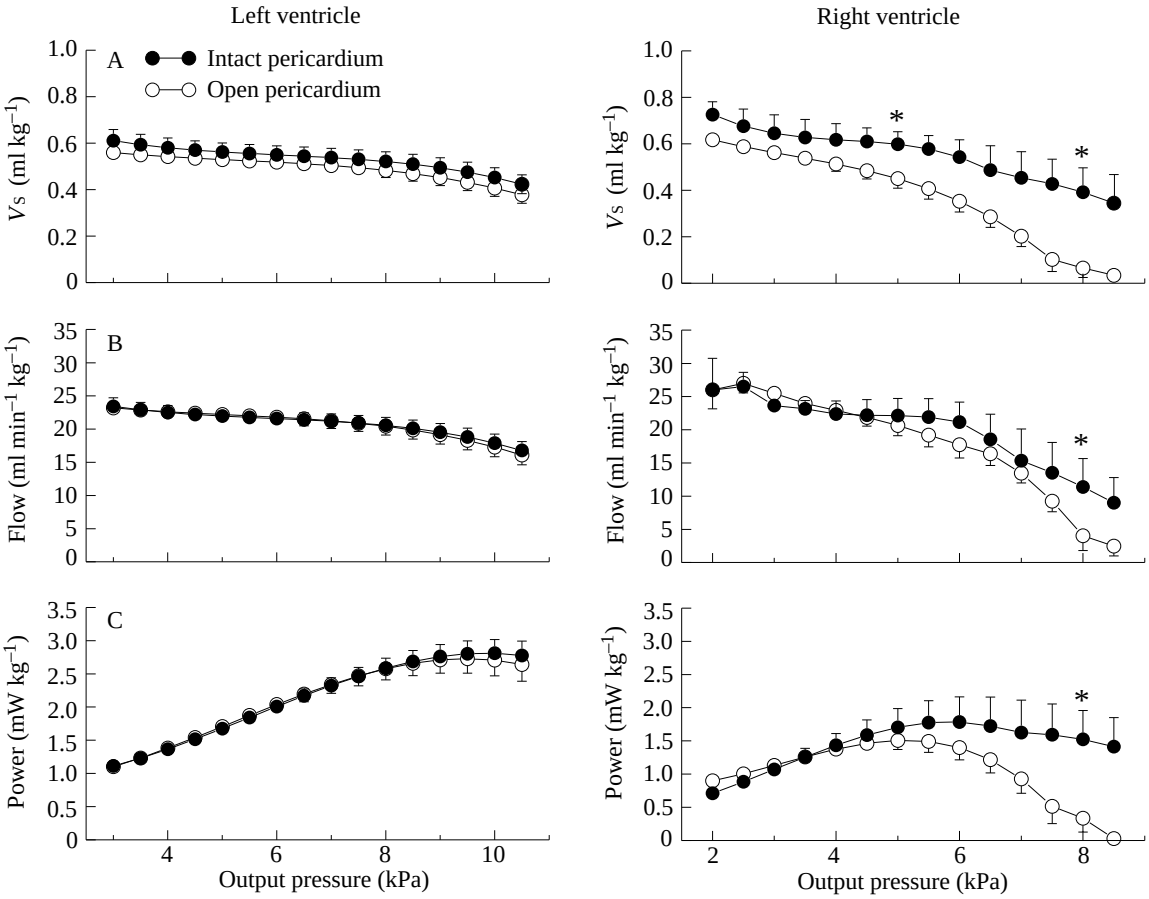

Fig. 7. Effect of opening the pericardium on the output pressure curves for the left and right ventricles. Filled circles represent the heart with an intact pericardium and the open circles the heart with the pericardium opened. Results are means ± S.E.M. (N=6). Asterisks indicates significant differences between the two conditions (P<0.05). V_S, stroke volume.

might be on the subpulmonary conus described by van Mierop and Kutsche (1985), where the cog-teeth-like valves are located. On the basis of phasic pressure and flow recordings, Shelton and Jones (1991) suggested that there might be a sudden opening of the subpulmonary outflow tract, and there is some evidence that β-adrenergic tone is important for the regulation of the flow resistance in this region (M. Axelsson and C. E. Franklin, unpublished observations).

At the lower P_{O_2} level, the ability of the heart to maintain stroke volume, ventricle flow and power output was reduced. Only at low work levels (output pressures) was the perfused *C. porosus* heart able to maintain stroke volume. A significant reduction in power output could be attributed to the intrinsic bradycardia which occurred with the decrease in P_{O_2}; the reduction in work which accompanies the bradycardia would have a protective effect on the heart.

Opening the pericardium did not affect the performance of the left ventricle in the face of increasing output pressure. The left ventricle was able to maintain its stroke volume. In contrast, the performance of the right ventricle, when exposed to output pressures in excess of 7 kPa, was reduced by opening the pericardium. Franklin and Davie (1991) also found that removal of the pericardium from the eel (*Anguilla dieffenbachii*) heart reduced the ability of the heart to maintain stroke volume against increasing output pressures. As in mammals, the pericardium in the crocodile appears to prevent over-stretching of the cardiac chambers, thus limiting the distension of the atria and ventricles. Being thinner-walled, the right ventricle would be more susceptible to over-stretching than the left ventricle, explaining the difference in the performance of the two ventricles after opening the pericardium.

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