THE SUB-PULMONARY CONUS AND THE ARTERIAL ANASTOMOSIS AS IMPORTANT SITES OF CARDIOVASCULAR REGULATION IN THE CROCODILE *CROCODYLUS POROSUS*

MICHAEL AXELSSON*, CRAIG E. FRANKLIN, REGINA FRITSCHE*, GORDON C. GRIGG
AND STEFAN NILSSON*,†

Department of Zoology, University of Queensland, Brisbane, Queensland 4069, Australia

Summary

We present evidence to support the hypothesis that the arterial anastomosis and the cogteeth-like valves located in the sub-pulmonary conus in the right ventricle are important sites of cardiovascular regulation in the crocodile *Crocodylus porosus*. The influence of the arterial anastomosis on the development of the 'foramen spike' in the left aortic pressure trace, which occurs at the onset of diastole when the pressures in the right and left aortas become equal, and on gastrointestinal blood flow was examined in unanaesthetised *C. porosus* using blood vessel occluders. Measurements of blood flow in the arterial anastomosis showed that, during non-shunting conditions, there was a substantial systolic blood flow from the right aorta into the coeliac artery. The total coeliac artery blood flow was the sum of the anastomosis flow from the right aorta plus the left aortic flow originating from the right aorta *via* the foramen of Panizza during diastole. During mechanically induced pulmonary-to-systemic shunting, the anastomosis blood flow was reversed, with blood flowing from the left to the right aorta. The magnitude of the 'foramen spike' was directly related to the vascular resistance in the anastomosis. When vascular resistance in the anastomosis was high, such as during mechanical occlusion, there was an increase in the right aortic to left aortic pressure gradient during systole which resulted in an increase the foramen spike amplitude. Recordings of right intraventricular pressure in unanaesthetised *C. porosus* showed spontaneous changes in right intracardiac systolic pressure. The pressure recordings were biphasic, with the second contraction (isometric) being highly variable in size, indicating the control of pulmonary outflow resistance, possibly *via* the 'cogteeth valves' located in the sub-pulmonary conus in the right ventricle.

Key words: cardiac physiology, circulatory control, foramen of Panizza, estuarine crocodile, *Crocodylus porosus*.

Introduction

The left (LAo) and right (RAo) aortas of crocodilians communicate at two points. The first is the foramen of Panizza (Panizza, 1833), an aperture in the inter-aortic septum just anterior to the aortic valves. The second is a vessel connecting the LAo and RAo, dorsal to the lungs, and is termed the arterial anastomosis. Posterior to the anastomosis, the LAo continues as the coeliac artery to the gut, while the RAo becomes the dorsal aorta (Fig. 1). It has been proposed that these two points in the cardiovascular system of crocodilians could exert considerable control over blood flow and distribution (Greenfield and Morrow, 1961; White, 1969; Grigg and Johansen, 1987; Grigg, 1989, 1991; Karila *et al.* 1995; Axelsson and Franklin, 1996). Another possible control site that has generated interest and that is unique to the crocodilians is the cogteeth-like valves located in the sub-pulmonary conus of the right ventricle (Webb, 1979; Jones and Shelton, 1993; Axelsson *et al.* 1995; Axelsson and Franklin, 1996). It has been suggested that these valves may influence pulmonary outflow resistance.

Studies investigating pressure and flow profiles in the LAo have attempted to provide some insight into the functional significance of this enigmatic vessel. Axelsson *et al.* (1989) published the first measurements of blood flows in unanaesthetised caimans, *Caiman crocodylus*. The net blood flow in the LAo was shown to be small and the flow profile complex, with both forward and backward blood flow during each cardiac cycle. These measurements of blood flow in the LAo confirmed blood pressure data which predicted that blood in the LAo emanates from the RAo *via* the foramen of Panizza during diastole and during a short period in early systole *via* the anastomosis (White, 1969; Grigg and Johansen, 1987; Axelsson *et al.* 1989). These complex flow patterns have been confirmed in the American alligator (*Alligator mississippiensis*) (Jones and Shelton, 1993; Malvin *et al.* 1995)

*Present address: Department of Zoophysiology, University of Göteborg, Medicinaregatan 18, S-413 90 Göteborg, Sweden.
†Author for correspondence (e-mail: S.Nilsson@zool.gu.se).

and in the Cuban crocodile (*Crocodylus rhombifer*) (Axelsson *et al.* 1995). The pressure and flow events that involve the 'round-the-loop' vasculature (i.e. the RAo–anastomosis–LAo pathway) are, however, less well understood, although a recent study has shown that the anastomosis has a well-developed adventitia and media with a dense innervation (Karila *et al.* 1995). It is apparent that measurement of flow in the anastomosis at rest and during shunting would permit a greater understanding of the role and function of the LAo.

The aim of the present study was to use *in vivo*, non-pharmacological methods to alter flows and pressures in the central vessels of *Crocodylus porosus* in order to study in more detail the pressure profiles in the left aorta and the flow of blood in the anastomosis in relation to coeliac artery blood flow and 'around-the-loop' effects (see Fig. 1). Surgically implanted vessel occluders allowed manipulations to be performed *in vivo* in fully recovered and unrestrained animals. Right intraventricular pressures were also recorded in unanaesthetised *C. porosus* to investigate the variable nature of the biphasic pressure pulse that occurs during systole (Jones and Shelton, 1993).

Materials and methods

Estuarine crocodiles, *Crocodylus porosus* (Schneider), were purchased from the Edward River Crocodile Farm, Queensland, Australia, and flown to Brisbane. The animals used in the experiments were of either sex, with a body mass of 3.4–6.6 kg. During the experiments, *C. porosus* were kept at 28–30 °C in a tank containing shallow water (20–30 cm) in which they could submerge at will.

Surgical procedures

In order to induce surgical anaesthesia, the glottis area was swabbed with lignocaine (20 mg ml^{-1}, Mavlab, Slacks Creek, Australia) and, after approximately 2 min, the animals were intubated using a close-fitting (5–6 mm) tracheal tube. During this procedure, the mouth was held open using a metal ring covered with a thick layer of rubber to avoid damage to the teeth. Anaesthesia was induced using halothane (Fluothane, Zeneca, UK) from an Ohmeda Fluotec 3 dispenser. A concentration of 4–5 % halothane in pure O_2 (medical grade) was used to induce a suitable level of anaesthesia, which was then maintained using 1–2 % halothane in pure O_2.

The animal was opened ventrally using a Surgistat (Valleylab) cautery knife which easily cut through the tough ventral skin of the crocodile, causing minimal bleeding. In order to gain access to the heart and major outflow vessels to place the flow probes and the pressure cannulae, it was necessary to part the sternum by 3–4 cm. The heart and outflow tract were then freed of surrounding tissue to expose the major arteries as they protrude from the anterior portion of the outflow tract (Fig. 1). After completion of implantation of probes and pressure cannulae (see below), the sternum was sutured together using interrupted sutures, and the ventral incision in the body wall and skin was also closed using sutures. Sterilised surgical equipment was used, and following surgery the animals

received daily injections of amoxycillin (approximately 30 mg kg^{-1} body mass, intramuscularly) to prevent infection.

After surgery, *C. porosus* were allowed to recover for at least 48 h before any experiments were conducted. In the few animals that underwent brief reoperation to change a malfunctioning flow probe or pressure cannula, at least 24 h was allowed for recovery before experiments were re-started.

Blood flow recordings

Blood flow recordings were made using a Doppler flow system (Iowa University), and in two crocodiles this was combined with an ultrasonic flow system (Transonic Systems Inc., model T206). For the Doppler flow recordings, custom-made cuff-type Doppler flow probes (diameter 2.5–3.5 mm), were placed around the RAo, the LAo and the left pulmonary artery immediately anterior to the outflow tract. In eight animals, the lobes of the liver were parted just posterior to the tip of the heart. The tissues surrounding the abdominal portions of the LAo and RAo include a common sheath of connective tissue. By carefully cutting this sheath open, the anastomosis can be reached and freed of the surrounding connective tissue sufficiently to allow a flow probe or occluder to be fitted on the vessel (see below). Under normal conditions, the diameter of the anastomosis is only slightly smaller than that of the coeliac artery, but during the clearing of the anastomosis and fitting of the flow probe, the vessel showed remarkable vasoactivity in that its diameter decreased dramatically.

The abdominal portions of the LAo and RAo, at the level of the anastomosis connecting the two, were located and freed of fat and connective tissue to allow the fitting of additional flow probes on the coeliac artery, on the LAo immediately proximal to the anastomosis or on the anastomosis itself. The Doppler probes were attached to a four-channel Doppler flow meter (Iowa University).

In two *C. porosus*, ultrasonic flow probes (diameter 2–4 mm) were placed on the RAo and LAo, or RAo and coeliac artery, respectively.

Blood pressure recordings

Blood pressure recordings were made using polyurethane cannulae (PU90; i.d. 0.9 mm, o.d. 1.2 mm) filled with heparinised (approximately 100 i.u ml^{-1}) 0.9 % NaCl, and non-occlusively implanted into the RAo, LAo and common pulmonary artery through the wall of the outflow tract with the opening downstream. In three crocodiles, right ventricular blood pressure was recorded (Fig. 1) using a similar cannula that had been perforated at the tip to prevent occlusion against the ventricle wall.

The polyurethane cannulae were attached to titanium injection ports (internal volume 0.17 ml) which were sutured onto the dorsal skin ridges of the crocodile, allowing needle-tipped polyethylene (PE90) cannulae to be attached during the experiments. These injection ports provided easy access to the pressure cannulae and minimised disturbance of the animals during experimentation but without affecting the frequency response of the cannulae/transducer system. The polyethylene
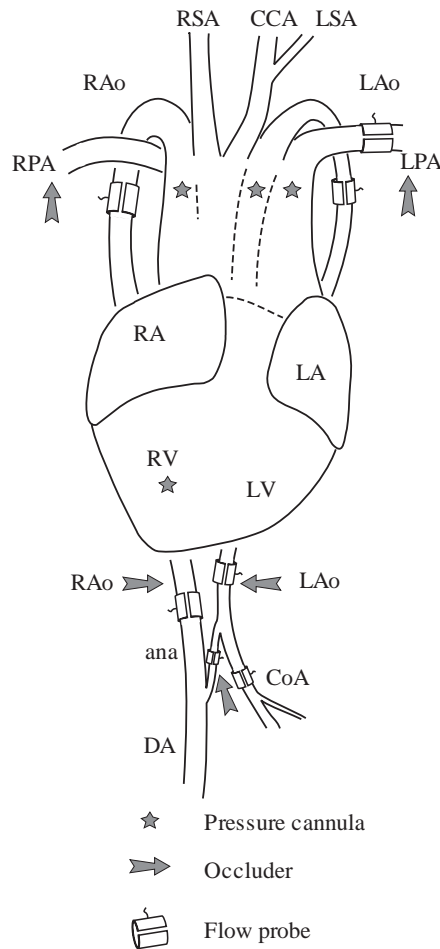
Fig. 1. A schematic representation of the crocodilian heart, showing the placement of pressure cannulae, occluders and flow probes. ana, anastomosis between left and right aorta; CCA, common carotid artery; CoA, coeliac artery; DA, dorsal aorta; LA, left atrium; LAo, left aorta; LPA, left pulmonary artery; LSA, left subclavian artery; LV, left ventricle; RA, right atrium; RAo, right aorta; RPA, right pulmonary artery; RSA, right subclavian artery; RV, right ventricle.

cannulae were, in turn, attached to Statham pressure transducers, which had been calibrated using a static column of water.

Mechanical occlusion of blood vessels

Hydraulic occluders were placed on the abdominal portions of the RAo or LAo, on the anastomosis or on the left and right pulmonary artery immediately distal to the outflow tract as indicated in Fig. 1. Note that while LAo occlusion effectively closes this vessel, occlusion of the RAo will still allow blood to flow into the subclavian and carotid arteries. Occluder cannulae were attached to dorsal injection ports, similar to the arrangement described above for the blood pressure cannulae. Water-filled 1 ml tuberculin glass syringes were used to inflate the occluder balloons.

Data acquisition, presentation and statistics

The flow meter(s) and the pressure transducers were connected to Grass Polygraph recorders (model 79), and heart

rate was derived from the pulsatile blood pressure or blood flow signal and displayed using a Grass model 7P4 or 7P44 tachograph preamplifier.

In addition to the Grass polygraph recordings, flow and pressure signals and heart rate (eight channels) were also sampled and recorded using a Toshiba 3200SX microcomputer running either AD/DATA (Dr Peter Thorén, Department of Physiology, University of Göteborg) or Lab-Tech Notebook software. Mean flow and pressure recordings were obtained using AD/DATA sampling at 5 samples s^{-1} and storing mean values at 12 points min^{-1}. High-speed sampling was achieved using LabTech Notebook at 100 samples s^{-1}.

Where applicable, statistical evaluation of the results was made using Wilcoxon signed rank tests. Statistical significance was set at $P<0.05$. Data are presented as means ± S.E.M.

Experimental protocol

After a recovery period of at least 48 h, crocodiles were monitored for heart rate, blood flow and pressure cycles. Occlusions were performed only after the water in the holding tank had been lowered to prevent voluntary submergence and the related circulatory changes that would obscure the direct effects of the occlusions. Each occlusion experiment was repeated several times on the same animal to check for variations in response, with at least 30 min recovery periods between repeats.

Results

General flow and pressure patterns

The recorded arterial pressures in *C. porosus* agree well with previously reported values for unanaesthetised crocodilian species, with a clear difference in mean pressure between the right/left aorta (6.2±0.4 kPa, N=9) and the pulmonary artery (2.1±0.1 kPa, mean ± S.E.M., N=8). There was also a significant difference between the right and left aortic pressure development during systole (Fig. 2, see also Figs 4, 6) (Axelsson *et al.* 1987, 1995; Jones and Shelton, 1993). The phasic flow patterns in the major vessels were also qualitatively similar to previously recorded flow patterns in unanaesthetised animals (see Figs 4, 5, 6) (Axelsson *et al.* 1987, 1995; Jones and Shelton, 1993; Karila *et al.* 1995). The left aortic flow profile showed the 'typical' three phases, with an initial anterograde flow followed by a retrograde flow which again reverses to an anterograde flow at the onset of diastole (see Figs 4, 5, 6) (Axelsson *et al.* 1987; Jones and Shelton, 1993; Karila *et al.* 1995). The phasic flow profile in the anastomosis is unusual, with a systolic 'flow-pulse' and close to zero diastolic flow (Figs 3, 4, see also Fig. 6 and below).

Right intraventricular pressure development showed a variable secondary isometric pressure peak (Fig. 4), which resulted in a variable pressure gradient between the right ventricle and the pulmonary artery with a maximum value of 3.3±0.5 kPa (N=3). These changes occurred spontaneously and over relatively short periods (see Fig. 4). In two of the three animals tested, there were periods with no secondary isometric pressure peak in the right ventricle and thus no pressure gradient between the ventricle and the pulmonary artery. There was a decrease in the amplitude of the second (isometric) pressure
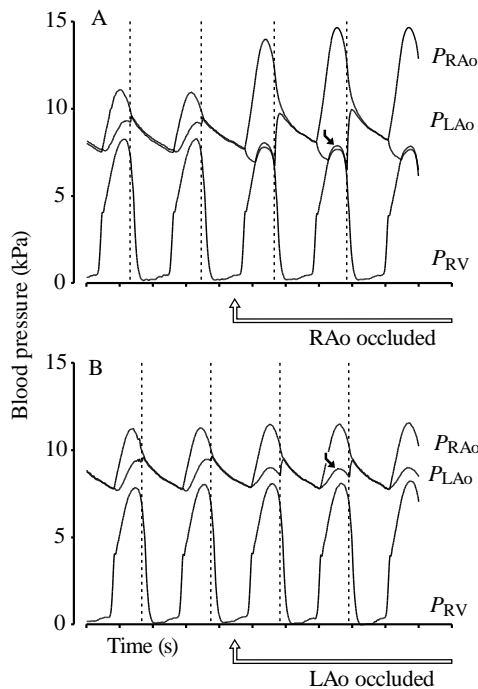
Fig. 2. Simultaneously recorded blood pressure profiles before and during mechanical occlusion of the right aorta (A, upper traces) or left aorta (B, lower traces). The start of occlusion is indicated by the open arrows. The end of systole is marked by vertical broken lines. Note especially the end-systolic 'foramen spike' in the P_{LAo} traces, which increases substantially during both RAo and LAo occlusion. During RAo occlusion, P_{LAo} falls off during systole, and P_{RAo} and P_{LAo} become synchronous at end-systole and during diastole. In this particular experiment, P_{LAo} falls low enough during systole to meet P_{RV}, forming a 'bump' in the P_{LAo} trace during a pulmonary-to-systemic shunt (solid arrow). During LAo occlusion, P_{LAo} increases during systole, but to a lesser degree than in the control situation (solid arrow). P_{LAo}, left aortic blood pressure; P_{RAo}, right aortic blood pressure; P_{RV}, right ventricular blood pressure.

peak with an increase in heart rate. For example, at a heart rate of 17.0 ± 3.5 beats min^{-1}, right ventricular pressure (P_{RV}) was 7.7 ± 0.7 kPa, whereas at a heart rate of 26.0 ± 3.2 beats min^{-1}, P_{RV} was only 5.9 ± 0.7 kPa (mean $\pm$ S.E.M., $N=3$; $P<0.05$).

Effects of mechanical occlusions

Occlusion of the LAo ($N=4$) (Figs 2, 3), the RAo ($N=4$) (Figs 2, 4) or the anastomosis ($N=1$) (Fig. 5) produced large changes in the left aortic pressure trace with a significant increase in the amplitude of the 'foramen spike', the largest changes occurring after RAo occlusion (Figs 2A, 4), while LAo or anastomosis occlusions resulted in almost identical changes (Figs 2B, 3, 5). Bilateral pulmonary artery occlusion ($N=3$) produced a significant increase in left aortic pressure (P_{LAo}), and the foramen spike in the left aortic pressure trace disappeared (Fig. 6). During RAo occlusion, P_{LAo} decreased throughout systole until right aortic pressure (P_{RAo}) and P_{LAo} become equal at the end of systole (Fig. 2A). Occasionally, systolic P_{LAo} decreased to meet peak P_{RV}, and a pulmonary-to-systemic shunt occurred (Fig. 2A). During LAo occlusion, and also
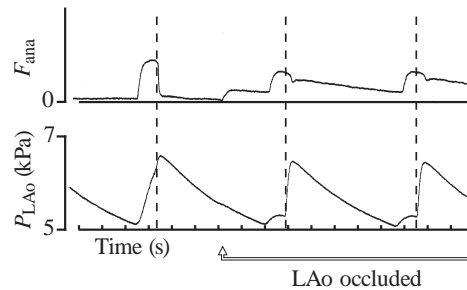

Fig. 3. Simultaneously recorded blood flow and pressure traces before and during mechanical occlusion of the left aorta (indicated by the open arrow). The end of systole is marked by vertical broken lines. Note especially the stop of F_{ana} during diastole before LAo occlusion, which is due to equalisation of the RAo and LAo blood pressures. Note also the very prominent 'foramen spike' at end-systole during occlusion, which reflects the opening of the foramen of Panizza. F_{ana}, anastomosis blood flow; P_{LAo}, left aortic blood pressure. F_{ana} is shown as the uncalibrated Doppler signal (ΔkHz Doppler shift), where upward pen deflection indicates right-to-left flow.

during occlusion of the anastomosis, there was an increase in P_{LAo} during systole, but this increase was significantly smaller than in the control situation (Figs 2A, 3, 5).

Direct measurements of blood flow in the anastomosis ($N=5$) revealed that blood flow was normally from the RAo to the LAo/coeliac artery during systole. During diastole, in the absence of a pressure gradient over the length of the anastomosis, flow was near zero (Figs 3, 4, 6). If, however, 'the loop' was obstructed, e.g. by occlusion of the LAo (Fig. 3) or RAo (Fig. 4), there was no diastolic pressure equilibrium between the RAo and LAo/coeliac artery, and the anastomosis flow therefore persisted throughout the cardiac cycle (reversed anastomosis flow; see Figs 3, 4).

Occlusion of the RAo produced a reversal of the anastomosis flow ($N=3$). Under these circumstances, the RAo systolic pressure is elevated, leading to an increase in right-to-left aortic blood flow through the foramen of Panizza, and this in turn results in a reversed (left-to-right) anastomosis flow (Fig. 4). Bilateral occlusion of the pulmonary arteries ($N=2$), simulating a total pulmonary-to-systemic shunt, also reversed the anastomosis flow (Fig. 6). This was not caused by an increase in the RAo systolic pressure but was due to a massive increase in left aortic blood flow (monophasic shunt; Shelton and Jones, 1993) and a simultaneous decrease in right aortic flow.

Occlusion of the anastomosis itself ($N=1$) produced changes in the LAo flow and pressure profiles, and in the coeliac artery blood flow (Fig. 5). The end-systolic pressure pulse in the LAo ('foramen spike') was much enhanced, as was the LAo diastolic flow pulse. The coeliac artery flow changed from the anterograde systolic flow pulse driven by P_{RAo} via the anastomosis to a lower flow with the peak at end-systole driven by the P_{LAo} and coinciding with the 'foramen spike' in the pressure trace. Direct absolute measurements of anastomosis flow were made using a Doppler flow probe. Simultaneous recording of the coeliac artery flow in one animal using an ultrasonic system made it possible to calibrate anastomosis

flow rate directly in absolute terms (Fig. 7). During LAo occlusion, the coeliac artery flow equals the anastomosis flow, and in this animal the flow through the anastomosis contributed approximately half the blood flow to the coeliac artery. Under control conditions, the rest of the flow was derived from the LAo from the RAo *via* the foramen of Panizza during diastole.

Discussion

This study details the effects of mechanical arterial occlusions (using novel cuff-like occluders) on the pressure and flow profiles in the major arteries of unanaesthetised *C. porosus*. Recordings of right intraventricular pressure development in *C. porosus* showed a systolic pressure gradient between the right ventricle and the pulmonary artery, confirming the work of Greenfield and Morrow (1961) and Jones and Shelton (1993) performed in anaesthetised *A. mississippiensis*. However, more important was the finding that the secondary isometric pressure peak was of variable size, indicating that there is a regulation of the outflow resistance between the right ventricle and the pulmonary artery (see Fig. 4).

In resting undisturbed *C. porosus*, P_{RV} never exceeded P_{LAo}, the LAo valves remaining closed. Jones and Shelton (1993)

reported spontaneously developing pulmonary-to-systemic shunts in unanaesthetised alligators. This may reflect differences in animal 'temperament' as the American alligator is known to be a generally even-tempered animal compared with *C. porosus*. This could influence the experimental systemic blood pressure and heart rate which, in turn, are factors that may induce shunting. During systole, there was a developing pressure difference between the right and left aorta, but at the onset of diastole, when the right aortic valve closes and the foramen of Panizza is uncovered, P_{LAo} and P_{RAo} equalise to produce a 'foramen spike' (Grigg and Johansen, 1987) of variable magnitude. The pressure difference between the LAo and RAo during systole shows that the foramen is indeed closed during systole owing to the obstruction caused by the opening of the medial cusp of the RAo valve, as suggested by Sabatier (1873). This was further verified by Axelsson *et al.* (1995) using angioscopy to observe the aortic valves directly in Ringer-perfused hearts of large specimens of the Cuban crocodile (*Crocodylus rhombifer*).

These results differ from the findings of White (1968, 1969) in the alligator (*A. mississippiensis*), where P_{RAo} and P_{LAo} were equal during the entire cardiac cycle. The discrepancies between these observations were partly clarified by Grigg and Johansen (1987), who observed that the pressure differences between the
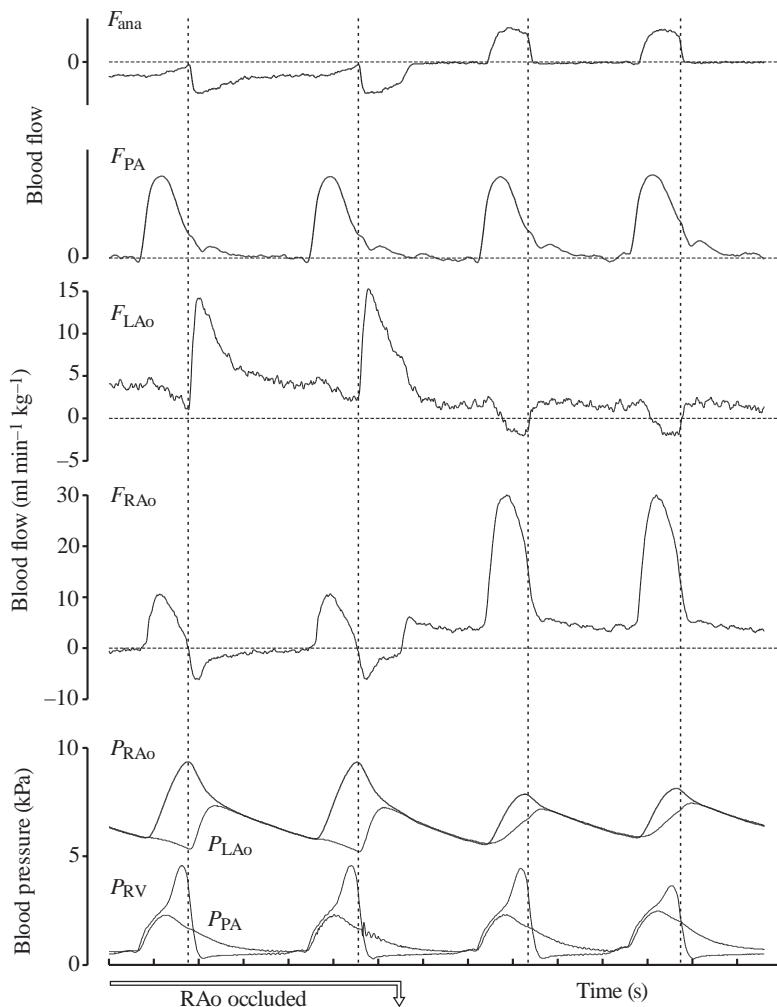

Fig. 4. Simultaneously recorded blood flow and pressure traces during (left part of traces) and after (right part of traces) mechanical occlusion of the right aorta (indicated by the open arrow). The end of systole is indicated by vertical broken lines. Note especially the reversal of anastomosis flow during occlusion. F_{ana}, anastomosis blood flow; F_{LAo} and F_{RAo}, left and right aortic blood flow; F_{PA}, pulmonary artery blood flow; P_{LAo}, left aortic blood pressure; P_{PA}, pulmonary artery blood pressure; P_{RAo}, right aortic blood pressure; P_{RV}, right ventricular blood pressure. F_{ana} and F_{PA} are shown as uncalibrated Doppler signals (ΔkHz Doppler shift), while F_{LAo} and F_{RAo} are calibrated (ml min^{-1} kg^{-1}) signals from the ultrasonic flow meter.
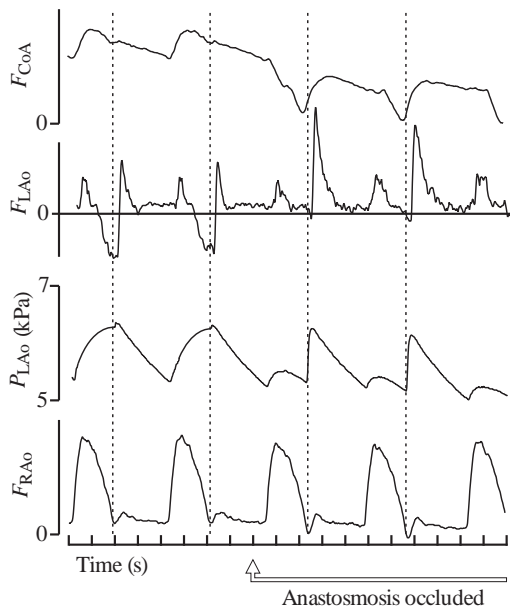
Fig. 5. Simultaneously recorded blood flow and pressure traces before and during mechanical occlusion of the anastomosis between the RAo and LAo (open arrow). The end of systole is indicated by vertical broken lines. Note especially the decrease in F_{CoA}, the lack of reversal in F_{LAo} and the very marked 'foramen spike' in the P_{LAo} trace at end-systole. F_{CoA}, coeliac artery blood flow; F_{LAo} and F_{RAo}, left and right aortic blood flow; P_{LAo}, left aortic blood pressure. F_{CoA}, F_{LAo} and F_{RAo} are shown as uncalibrated Doppler signals (ΔkHz Doppler shift), while P_{LAo} is calibrated (kPa).

right and left aorta of *C. porosus* were associated with high heart rate and high systemic blood pressure, while during periods of low systemic blood pressure the right and left aortic pressures were equal during the entire cardiac cycle. Blood pressure alone may not explain this phenomenon since some of the alligators in the study by White (1968) had high blood pressures and still showed overlapping P_{RAo} and P_{LAo} values during the entire cardiac cycle. The histochemical studies by Karila *et al.* (1995) on the foramen of Panizza suggest a moderately dense innervation of the foramen circumference. This observation, in combination with the arrangement of the muscle around the foramen circumference, is compatible with the view of a variable foramen diameter as suggested by Grigg and Johansen (1987). This may help explain the discrepancies between these studies in the pressure development in the right and left aorta. The circumstances leading to changes in foramen diameter remain, however, unknown.

The presence of a 'foramen spike', i.e. a rapid end-systolic increase in P_{LAo} when the foramen is uncovered and when P_{LAo} equilibrates with the higher P_{RAo}, has been observed by several workers (Greenfield and Morrow, 1961; Grigg and Johansen, 1978; Axelsson *et al.* 1989; Shelton and Jones, 1991; Jones and Shelton, 1993), but was dismissed by Shelton and Jones (1991) as being due to an experimental artefact introduced by partial obstruction of 'the loop', i.e. the connection of the RAo and LAo *via* the anastomosis by the inserted pressure cannula. Indeed, as shown in the present study, obstruction of this 'loop'
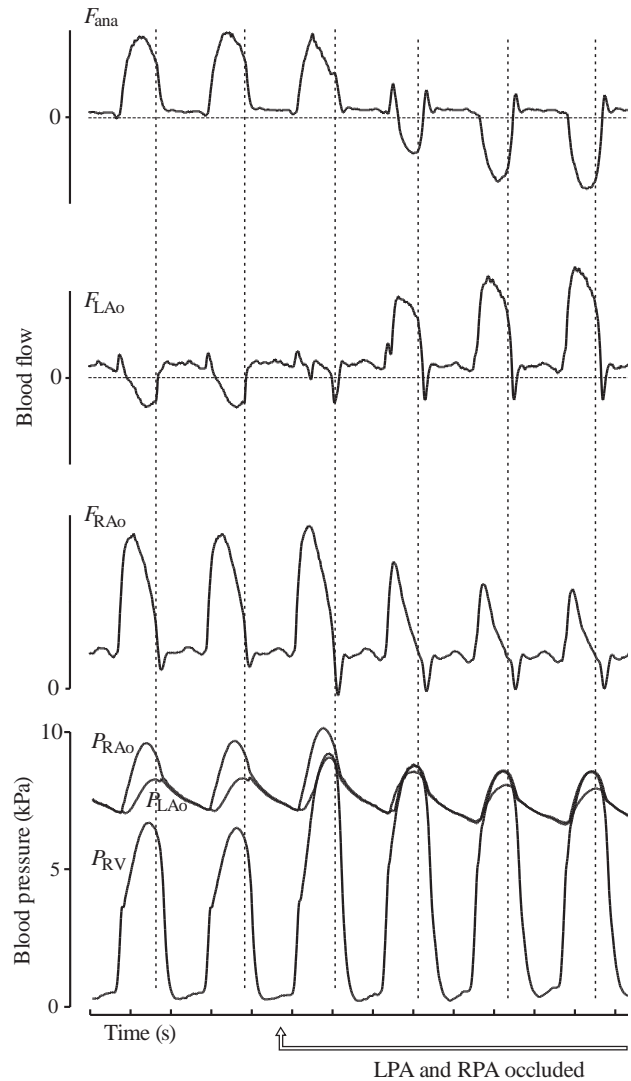

Fig. 6. Simultaneously recorded blood flow and pressure traces before and during bilateral mechanical occlusion of the pulmonary arteries (LPA and RPA, indicated by the open arrow). The end of systole is indicated by vertical broken lines. Note especially the reversal of the left aortic and anastomosis flow. Blood flows are shown as uncalibrated Doppler signals (ΔkHz Doppler shift). F_{ana} anastomosis blood flow; F_{LAo} and F_{RAo}, left and right aortic blood flow; P_{RAo} and P_{LAo}, left and right aortic blood pressure; P_{RV} right intraventricular pressure; LPA and RPA, left and right pulmonary arteries.

by left aortic or anastomosis occlusion produces an increase in the RAo-to-LAo pressure gradient during systole, thus enhancing the end-systolic 'foramen spike'.

Axelsson *et al.* (1995) found evidence for a small early systolic blood flow from the RAo through the foramen. This flow occurred before the foramen was completely covered by the medial cusp of the RAo valve. It is unlikely that this small flow pulse is the sole cause of the systolic pressure increase in the left aorta. Occlusion of the left aorta or anastomosis showed that part of the systolic pressure increase in the left aorta was due to an 'around-the-loop' effect (Jones and Shelton, 1993). The remaining pressure increase must therefore be a combination of
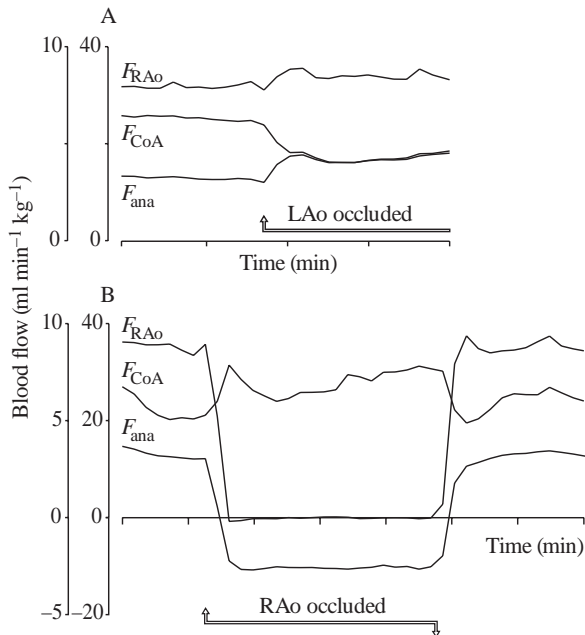
Fig. 7. Simultaneously recorded blood flow traces during mechanical occlusion (open arrows) of the left aorta (A, top traces) and right aorta (B, bottom traces). Calibration of F_{ana} and F_{CoA} are shown on the left-hand y-axis, while the right-hand y-axis refers to F_{RAo}. F_{ana}, anastomosis blood flow; F_{CoA}, coeliac artery blood flow; F_{RAo}, right aortic blood flow. Flow in the anastomosis (F_{ana}) was measured using a Doppler flow meter, and the volume flow was calibrated during occlusion of the left aorta when $F_{ana}=F_{CoA}$ (top traces). Note the reversal of anastomosis flow during RAo occlusion.

the small flow pulse and the transmural pressure pulse from the adjacent vessels within the outflow tract (notably the RAo).

Under control conditions, the size of the 'foramen spike' was quite variable in our experiments, even within the same animal. This could be explained by assuming that the vascular resistance around 'the loop' could be varied, for example by changes in the diameter of the anastomosis. In favour of this idea is the observation of a heavily innervated muscular wall in the anastomosis (Karila *et al.* 1995), as well as our own observations of rapid and marked changes in anastomosis diameter during surgery. Right aortic occlusion, just anterior to the anastomosis, produced an increase in right aortic pressure and a decrease in left aortic pressure that was large enough for a pulmonary-to-systemic shunt to develop; it also produced the most spectacular foramen spike in the left aortic pressure trace (Fig. 2A). During this procedure, the right aortic blood flow into the dorsal aorta was zero, and therefore the driving pressure for the dorsal aortic flow was the left aortic pressure driven by the small diastolic foramen flow (Fig. 7B). This, in turn, induced the fall in the left aortic pressure, initiating a pulmonary-to-systemic shunt (Fig. 2A).

Pulmonary artery pressure profile

A marked pressure difference between the right ventricle and the pulmonary artery has been described in anaesthetised

crocodilians (e.g. Greenfield and Morrow, 1961; White, 1969; Grigg and Johansen, 1987; Axelsson *et al.* 1989; Shelton and Jones, 1991; Jones and Shelton, 1993). The same pressure development in the right ventricle was found in our unanaesthetised unrestrained animals, but we could also see spontaneous changes in the amplitude of the second pressure peak indicating active control of the resistance between the right ventricle and the pulmonary circuit (see Fig. 4). Interestingly, there was a correlation between heart rate and the size of the pressure difference, with larger pressure gradients occurring at low heart rates. In a study by Malvin *et al.* (1995), vagal stimulation lowered the heart rate and maintained or produced a pulmonary-to-systemic shunt. In an angioscopic study (Axelsson *et al.* 1995), the connective tissue outpushings in the sub-pulmonary conus (Webb, 1979) were clearly observed, during systole, to move together like 'the teeth of opposing cogs' (Webb, 1979), completely obstructing the flow into the pulmonary arteries. It therefore seems very likely that the limitation of pressure development in the pulmonary arteries is due directly to this closure of the pulmonary outflow tract by the cogteeth and that the movement of these structures can be controlled actively.

Anastomosis flow

In a previous study, we observed that the blood flow pattern in the coeliac artery of resting *C. porosus* resembled more closely that of the RAo than that of the LAo, and we concluded that the coeliac artery blood was derived largely from the RAo *via* the anastomosis (Axelsson *et al.* 1991). This conclusion was confirmed in the present work by direct measurements of the anastomosis blood flow, and it was also possible to demonstrate a systolic flow from the RAo to the LAo through the anastomosis. The unusual flow profile seen in the anastomosis (Figs 3, 4, 6), with a 'pulse' of blood during systole and a close to zero diastolic flow, is due to the presence of the foramen of Panizza. During systole, when the foramen is covered, blood will flow through the anastomosis because of the increase in pressure in the right aorta relative to the left/coeliac artery, but at the onset of diastole the foramen is uncovered and pressure equilibration between the left and right aorta occurs, reducing the driving pressure for the anastomosis flow to zero.

Proposed functional significance

From the *in vivo* measurements of the right intraventricular pressure development during the cardiac cycle, we are confident that the variable magnitude of the second (isometric) contraction phase indicates close control of the resistance of the sub-pulmonary conus and thus the resistance to flow into the pulmonary circulation. This is in line with the proposals of Jones and Shelton (1993). We also present results in support of the hypotheses put forward by Grigg and Johansen (1987) that the pressure profiles observed in the LAo pressure trace can be attributed in part to a pressure pulse reaching the LAo 'round-the-loop', i.e. from the RAo *via* the anastomosis. Part of this transient pressure event is a combination of a small foramen flow, as reported by Axelsson *et al.* (1995), and the pressure pulse acting transmurally within the outflow tract.

Studies of the function of the anastomosis suggest that the gastrointestinal blood flow under normal conditions is derived to a large extent from the RAo *via* the anastomosis. Changes in the vascular resistance of the anastomosis could affect gastrointestinal blood supply, but we do not think that this is the main function of the anastomosis. Karila *et al.* (1995) showed that regulation of the gastrointestinal vascular resistance can be independent of the anastomosis, indicating other functions for this short muscular arterial segment. One of the possible functions of the anastomosis/left aorta could be to initiate a pulmonary-to-systemic shunt after feeding. The systemic vascular resistance, one of the important factors in the control of the pulmonary-to-systemic shunt (Axelsson *et al.* 1989; Jones and Shelton, 1993), is composed of many different vascular circuits. Among these, the gastrointestinal vasculature is one of the larger components and can be further subdivided into the coeliac and mesenteric vascular circuits. In crocodilians, the coeliac artery is the continuation of the left aorta, while the mesenteric artery is derived from the dorsal aorta. An increase in the vascular resistance of the anastomosis would isolate the coeliac vascular circuit from the right/dorsal aorta and, in a post-prandial state when the coeliac vascular resistance is lowered, this would lead to a fall in the left aortic pressure and eventually a shunt. Jones and Shelton (1993) speculated about the potential benefits arising from shunting acidic blood to the gut during periods of high HCl secretion. In the present study, the animals were fasted and the coeliac vascular resistance was probably high. However, as shown in Fig. 2A, when the right aorta was occluded, the right aortic pressure increased while the left aortic pressure decreased because of blood draining into the dorsal aorta and coeliac artery leading to the development of a pulmonary-to-systemic shunt. Another possible functional significance of the anastomosis is to prevent blood flow from the LAo to the RAo (dorsal aorta) during pulmonary-to-systemic shunting. During pulmonary-to-systemic shunting, when the left ventricular pressure and flow generation are reduced as a result of lowered venous return, the carotid and coronary circulation could receive blood from the LAo *via* the foramen of Panizza ('reversed foramen flow'; Grigg, 1991; Axelsson *et al.* 1995). By concomitant increases in the resistance of the anastomosis, thus preventing LAo-to-RAo 'backflow' *via* the anastomosis, the driving pressure of the 'reversed foramen flow' would be secured. Our observations lend some support to the hypothesis that the vascular resistance of the anastomosis can change substantially, but the circumstances leading to such changes remain to be elucidated.

References

Axelsson, M. and Franklin, C. E. (1996). From anatomy to angioscopy, 163 years of crocodilian cardiovascular research, recent advances. *Comp. Biochem. Physiol.* (in press).

Axelsson, M., Franklin, C. E., Löfman, C. O., Nilsson, S. and Grigg, G. C. (1995). Dynamic anatomical study of cardiac shunting in crocodiles using high-resolution angioscopy. *J. exp. Biol.* **199**, 359–365.

Axelsson, M., Fritsche, R., Holmgren, S., Grove, D. J. and Nilsson, S. (1991). Gut blood flow in the estuarine crocodile, *Crocodylus porosus. Acta physiol. scand.* **142**, 509–516.

Axelsson, M., Holm, S. and Nilsson, S. (1989). Flow dynamics of the crocodilian heart. *Am. J. Physiol.* **256**, R875–R879.

Greenfield, L. J. and Morrow, A. G. (1961). The cardiovascular hemodynamics of Crocodilia. *J. surg. Res.* **1**, 97–103.

Grigg, G. C. (1989). The heart and patterns of cardiac outflow in Crocodilia. *Proc. Aust. Physiol. Pharmac. Soc.* **20**, 43–57.

Grigg, G. C. (1991). Central cardiovascular anatomy and function in Crocodilia. In *Physiological Adaptations in Vertebrates: Respiration, Circulation and Metabolism. Lung Biology in Health and Disease*, vol. 56 (ed. S. C. Wood, R. E. Weber, A. R. Hargens and R. W. Millard), pp. 339–354. New York: Marcel Dekker.

Grigg, G. C. and Johansen, K. (1987). Cardiovascular dynamics in *Crocodylus porosus* breathing air and during voluntary aerobic dives. *J. comp. Physiol.* **157**, 381–392.

Jones, D. R. and Shelton, G. (1993). The physiology of the alligator heart: left aortic flow patterns and pulmonary-to-systemic shunts. *J. exp. Biol.* **176**, 247–269.

Karila, P., Axelsson, M., Franklin, C. E., Fritsche, R., Gibbins, I. L., Grigg, G. C., Nilsson, S. and Holmgren, S. (1995). Neuropeptide immunoreactivity and co-existence in cardiovascular nerves and autonomic ganglia of the estuarine crocodile, *Crocodylus porosus* and cardiovascular effects of neuropeptides. *Regulatory Peptides* **58**, 25–39.

Malvin, G. M., Hicks, J. W. and Green, R. E. (1995). Central vascular flow patterns in the alligator *Alligator mississipiensis. Am. J. Physiol.* **269**, R1133–R1139.

Panizza, B. (1833). Sulla struttura del cuore e sulla circolazione del sangue del *Crocodilus lucius. Biblioth. Ital.* **70**, 87–91.

Sabatier, A. (1873). Etudie sur le coeur et la circulation centrale dans la série des vertebrés. *Ann. Sci. nat. Zool. Ser.* V **18**, 1–89.

Shelton, G. and Jones, D. R. (1991). The physiology of the alligator heart: the cardiac cycle. *J. exp. Biol.* **158**, 539–564.

Webb, G. J. W. (1979). Comparative cardiac anatomy of the Reptilia. III. The heart of crocodilians and a hypothesis on the completion of the interventricular septum of crocodilians and birds. *J. Morph.* **161**, 221–240.

White, F. N. (1968). Functional anatomy of the heart of reptiles. *Am. Zool.* **8**, 211–219.

White, F. (1969). Redistribution of cardiac output in the diving alligator. *Copeia* **3**, 567–570.