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Delayed depolarization of the cog-wheel valve and pulmonary-to-systemic shunting in alligators

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Summary

Alligators and other crocodilians have a cog-wheel valve located within the subpulmonary conus, and active closure of this valve during each heart beat can markedly and phasically increase resistance in the pulmonary outflow tract. If this increased resistance causes right ventricular pressure to rise above that in the systemic circuit, right ventricular blood can flow into the left aorta and systemic circulation, an event known as pulmonary-to-systemic shunting. To understand better how this valve is controlled, anaesthetized American alligators (*Alligator mississippiensis*) were used to examine the relationships between depolarization of the right ventricle, depolarization/contraction of the cog-wheel valve muscle and the resultant right ventricular, pulmonary artery and systemic pressures. Depolarization swept across the right ventricle from the apex towards the base (near where the cog-wheel valve muscle is located) at a velocity of 91±23 cm s^{-1} (mean ± S.E.M., *N*=3). The cog-wheel valve electrocardiogram (ECG) (and thus contraction of the valve) trailed the right ventricular ECG by 248±28 ms (*N*=3), which was equivalent to 6–35 % of a cardiac cycle. This long interval between right ventricular and valve depolarization suggests a nodal delay at the junction between the base of the right ventricle and the cog-wheel valve. The delay before valve closure determined when the abrupt secondary rise in right ventricular pressure occurred during systole and is likely to strongly influence the amount of blood entering the pulmonary artery and thus to directly control the degree of shunting. Left vagal stimulation (10–50 Hz) reduced the conduction delay between the right ventricle and cog-wheel valve by approximately 20 % and reduced the integrated cog-wheel ECG by 10–20 %. Direct application of acetylcholine (1–2 mg) also reduced the integrated cog-wheel ECG by 10–100 %; however, its effect on the conduction delay was highly variable (–40 to +60 %). When the cog-wheel valve muscle was killed by the application of ethanol, the cog-wheel ECG was absent, right ventricular and pulmonary pressures remained low and tracked one another, the secondary rise in right ventricular pressure was abolished and shunting did not occur. This study provides additional, direct evidence that phasic contraction of the cog-wheel valve muscle controls shunting, that nervous and cholinergic stimulation can alter the delay and strength of valve depolarization and that this can affect the propensity to shunt.

Key words: alligator, *Alligator mississippiensis*, blood pressure, cardiac muscle, heart, shunt, left aorta, pulmonary artery, right ventricle, electrocardiogram, cog-wheel valve, conduction velocity, nodal delay.

Introduction

The crocodilian ventricle is unique among reptiles because it is morphologically divided into left and right halves by a complete interventricular septum. Although this anatomical design prevents the mixing of systemic and pulmonary venous blood within the ventricle, blood can still cross between the circuits *via* several routes outside the heart. The right ventricle (RV) maintains a connection to the systemic circulation *via* the left aorta (LAo, Fig. 1), and blood flow from the right ventricle, through the LAo and into the systemic circuit is known as a pulmonary-to-systemic (P→S) shunt (Jones, 1996). In addition, blood can be exchanged between the LAo and the right aorta (RAo, arising from the left ventricle) through the foramen of Panizza, which is located just downstream of the ventricular valves, and *via* an anastomosis (JJ) between the two vessels behind the heart (Fig. 1).

Crocodilians also possess a well-developed and muscular

cog-wheel valve located in the subpulmonary conus just outside the RV (Fig. 1) (Greenfield and Morrow, 1961; Webb, 1979; Farrell et al., 1998). This valve consists of connective tissue nodules that fit together to partially or completely occlude the conus; it is surrounded by a mass of cardiac muscle (van Mierop and Kutsche, 1985). Contraction of this muscle causes closure of the valve and increased pulmonary input resistance and would account for the phasic and complex alterations in RV pressure that occur with each cardiac cycle and cause P→S shunting (Greenfield and Morrow, 1961; White, 1969, 1970; Grigg and Johansen, 1987; Axelsson et al., 1989, 1996; Shelton and Jones, 1991; Jones and Shelton, 1993; Jones, 1996).

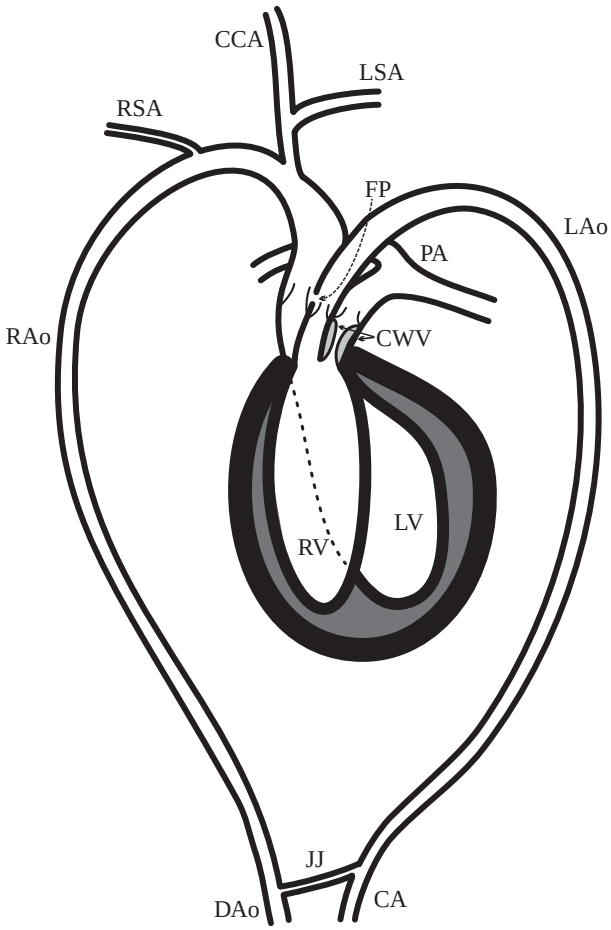

Fig. 1. The crocodilian central circulation, ventral view. The right ventricle (RV) maintains connections to both the pulmonary circuit *via* the pulmonary artery (PA) and the systemic circuit *via* the left aorta (LAo), which continues as the coeliac artery (CA). The subpulmonary conus contains a muscular cog-wheel valve (CWV, made of cartilagenous teeth and surrounded by cardiac muscle), the contraction of which can occlude the entrance to the pulmonary artery. The right aorta (RAo) receives blood from the left ventricle (LV), gives rise to the common carotid artery (CCA) and the right/left subclavian arteries (R/LSA) and then continues as the dorsal aorta (DAo). The left and right aortas connect twice, just outside the ventricles through the foramen of Panizza (FP) and behind the heart *via* an anastomosis (JJ).

In crocodilians, the merits of P→S shunting and the mechanism(s) by which shunts are controlled are not well understood (for reviews, see Jones, 1996; Burggren, 1987). Franklin and Axelsson (2000), using an isolated heart model in which the pulmonary outflow tract had been removed, showed that β-adrenergic stimulation reduces resistance in the subpulmonary conus, reduces RV pressure development and thus inhibits P→S shunting in the crocodile. In addition, Axelsson and Franklin (2001) show that the calibre of the foramen of Panizza in the aortic outflow tract is variable and subject to adrenergic constriction, which will have consequences for flow patterns in the left and right aortas during shunting. However, sustained adrenergic tonus cannot account for the aforementioned phasic and complex changes in RV pressure seen in crocodilians late in the cardiac cycle. Thus, there must also be large and phasic changes in resistance in the pulmonary outflow tract with each heart beat. Franklin and Axelsson (2000) and Axelsson and Franklin (2001) make no claim that an adrenergically mediated mechanism causes phasic changes in resistance within each cardiac cycle; indeed, such changes occurred with a time course of minutes in their experiments. Hence, some other mechanism(s) must also be responsible for regulating this valve and shunting in crocodilians.

The phasic changes in resistance suggest phasic activity of the cog-wheel valve during each cardiac cycle and strongly suggest that depolarization of the muscle mass surrounding the valve is linked to RV contraction. White and Brady (unpublished observation cited in White, 1968) noted a 350 ms delay between depolarization ‘near the pulmonary artery’ and the adjacent ventricle in alligators, and proposed that this may be the source of the phasic increase in resistance between the RV and pulmonary artery (PA). Further, Burggren’s (1978) work on turtle hearts implies a 200–300 ms delay mechanism linking depolarization in the main ventricle to that in the bulbus surrounding the PA. There are no published reports that describe a link between depolarization of the RV and of the cog-wheel valve muscle in crocodilians nor the phasic relationships between valve depolarization and the central pressure gradients causing P→S shunting. In this paper, we present evidence that cog-wheel valve activity is synchronized with RV depolarization through a nodal delay mechanism and that the phasic relationships that exist between RV contraction and valve activity are a key mechanism allowing crocodilians to regulate shunting on a beat-by-beat basis.

Materials and methods

Experiments were conducted at the University of British Columbia, and all procedures conformed to UBC animal care guidelines. American alligators (*Alligator mississippiensis*) of both sexes were obtained from commercial farms in Florida, USA, and held in the animal care facilities at the university. Animals were housed individually or in small groups in rooms (approximately 15 m^2, 30 °C) containing a shallow pool in which they could submerge. They were fed commercial dog

chow *ad libitum* and given chopped chicken and vitamin supplements weekly. All animals were growing and appeared healthy before these experiments. Recordings were made on 11 animals; mass range 8.6–37.5 kg, mean 21.5±2.5 kg (mean ± S.E.M.). Because not all recordings could be made on every animal, the number of alligators used for each set of measurements varied. Data in the text, figures and table are expressed as means ± S.E.M. Linear regressions and correlations were used to describe relationships between heart rate, electrocardiogram (ECG) timing and conduction velocity and delay, with $P<0.05$ considered significant.

Anaesthesia and instrumentation

Animals were initially sedated by injecting 25 mg kg^{-1} Ketamine HCl (Ketalean, Bimeda-MTC, Cambridge, Ontario, Canada) into the tail musculature, and then placing a mask containing a Halothane-wetted cloth over their nostrils (MTC Pharmaceuticals, Cambridge, Ontario, Canada). When the animals had been sufficiently sedated, they were weighed and placed supine on a surgical table. They were then intubated and ventilated with a small-animal ventilator, modified for use on alligators, and brought to surgical anaesthesia with 3–4 % Halothane in 1:1 N_2O:O_2. A catheter was placed in the right femoral vein, and infusion of Ketamine (5–15 mg^{-1} kg^{-1} h^{-1}) was initiated. Halothane and N_2O anaesthesia were suspended at this point, but forced ventilation with 100 % oxygen was continued. A heating pad was used to maintain core body temperature at 30 °C throughout the experiments (monitored using a rectal probe).

Xylocaine (2 % lidocaine HCl, Astra Pharma Inc., Mississauga, Ontario, Canada) was then injected subcutaneously along the ventral midline, and an incision was made through the skin and ribs to expose the heart and central vasculature. The heart was exposed by slitting the pericardial sac along the rostro-caudal midline. Silk sutures were tied to the cut edges of the pericardium and secured to a ring stand placed above the heart. This formed a bath around the heart that was filled with mineral oil to prevent desiccation and to reduce the bulk flow of current around the heart. A non-occlusive pressure catheter (Bolab medical vinyl tubing, Lake Havasu City, Arizona, USA), pre-treated with an anticoagulating agent (TD-MAC, Polysciences Inc., Warrington, Pennsylvania, USA), was inserted into the right subclavian artery. The tip of a 16-gauge hypodermic needle was fixed to a second pressure catheter and inserted into the pulmonary artery. A third catheter, similarly fashioned, was inserted directly into the right ventricle through the ventricular wall. Catheters were filled with degassed, heparinized (40 i.u. ml^{-1}), 0.9 % NaCl saline, cleared of bubbles and connected to Deltran II pressure transducers (Utah Medical Products, Midvale, Utah, USA). These transducers were routinely calibrated against a mercury column during experiments.

Bipolar ECG electrodes (1 mm tip spacing) were made from 44-gauge copper magnet wire and chemically sharpened. One was inserted into the main RV muscle mass, and the other was inserted into the centre of the mass of muscle surrounding the cog-wheel valve. ECG electrodes were connected to Gould isolated preamplifiers (model 11-5407-58) and Gould universal amplifiers (model 13-4615-58) with a 3 Hz to 1 kHz band-pass. The ECG data were digitally filtered offline using a high-pass finite impulse response (FIR) filter (10–30 Hz cut-off) to remove movement artifacts. All pressure and ECG data were collected on a computer using LabTech Notebook Pro v9 software (Labtech, Andover, Massachusetts, USA).

ECG recordings

Propagation of the ECG across the RV and into the muscle surrounding the cog-wheel valve was recorded by positioning one electrode in the cog-wheel muscle (located approximately 1 cm from the base of the RV) and a second electrode at different locations on the RV (11–15 different locations in each of three animals). Conduction velocity across the RV was calculated as the slope of the regression relating the distance between electrodes and conduction time. The intercept of this relationship was the conduction delay that occurred at the junction of the RV and cog-wheel valve muscle (nodal delay).

Experimental manipulations

After recording the RV conduction velocity and delay, the effects of parasympathetic and cholinergic stimulation on central pressures, ECGs and cog-wheel valve function were studied. ECG electrodes were placed in the middle of the RV and in the muscle mass surrounding the cog-wheel valve. Pressures from two of the three catheters (RV, subclavian, PA) and both ECGs were measured under ‘control’ conditions (no manipulations), when cog-wheel valve contraction and heart rate were modified by direct application of acetylcholine (ACh) to the muscle surrounding the cog-wheel valve or to the RV muscle (see Table 1 for details), during vagal stimulation or after cog-wheel valve function had been temporarily weakened by injecting Xylocaine or the valve had been killed by injection of 95 % ethanol into the cog-wheel valve muscle. Vagal stimulation was successful in two of the three animals in which it was attempted; we report results from only the two successful experiments. In these animals, a cuff electrode (custom-made) was placed around the isolated left vagus nerve, and the stimulation frequency was set at 10, 20 or 50 Hz (50 μs pulse duration and 5–7 V amplitude). Changes in pressure profiles, heart rate, the conduction delay between the RV and cog-wheel ECGs, the phase of the cog-wheel ECG (see below) and the integrated cog-wheel ECG (see below) were measured after these manipulations. These experimental recordings were bracketed by control recordings, although it was not possible to bracket experiments in which the cog-wheel muscle was killed with ethanol.

Phase was defined as the percentage ratio of the conduction delay to the duration of a cardiac cycle (i.e. the percentage of a cardiac cycle that elapsed between the RV ECG and the cog-wheel ECG). The integrated cog-wheel ECG was used as a measure of the strength of the cog-wheel depolarization or the

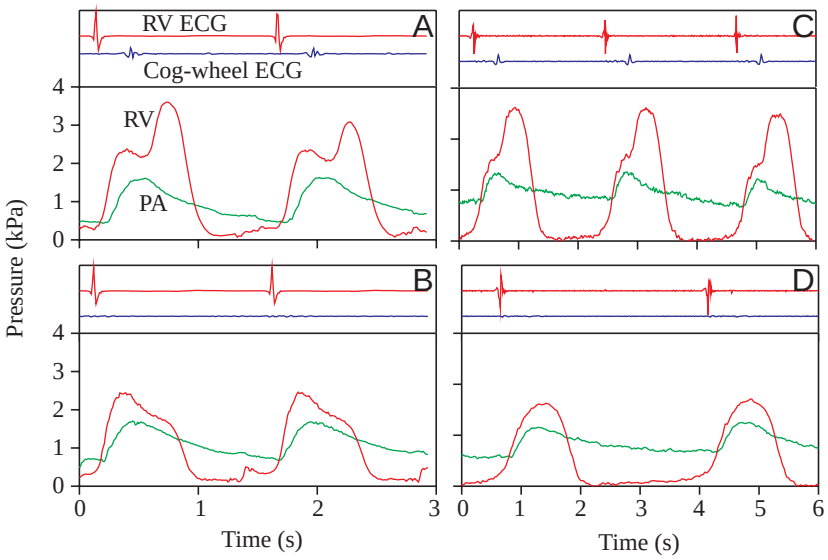

Fig. 2. Electrocardiograms (ECGs) and pressures in the alligator pulmonary circuit. Top traces, ECGs recorded from the middle of the right ventricle (RV ECG, red) and the cog-wheel valve muscle (cog-wheel ECG, blue). Bottom traces, right ventricular pressure (RV, red) and pulmonary arterial pressure (PA, green). A and C show results from animals with a functioning cog-wheel valve. B and D show results from the same animals, but after the cog-wheel valve had been inactivated by application of acetylcholine.

muscle's activity; the filtered cog-wheel ECGs were rectified, and the voltage/time integral was subsequently measured over the period of the ECG. All calculations were made using AcqKnowledge software (v3.01 BIOPAC Systems, Inc., Santa Barbara, California, USA). At the conclusion of each experiment, the animals were killed with an intracardiac overdose of pentobarbital, and the hearts and central vessels were dissected to confirm the appropriate placement of all pressure catheters.

Results

ECGs, central blood pressures and elimination of cog-wheel valve function

Recordings of RV and cog-wheel valve muscle ECGs, RV pressure, systemic pressure (subclavian) and PA pressure are shown in Figs 2 and 3. The RV ECG was coincident with a rise in RV pressure, and increases in PA pressure were observed when RV pressure equalled or exceeded diastolic PA pressure (i.e. the pulmonary valve opened). Coincident with the cog-wheel muscle ECG was a large, secondary rise in RV pressure and an uncoupling of PA pressure from that in the RV (Figs 2A,C, 3A). Activation of the cog-wheel valve often caused an almost doubling of the pressure developed by the RV. This marked increase in RV pressure was sufficient to meet or exceed that in the systemic circulation in many instances (Fig. 3B) and would favour the ejection of RV blood into the LAo and thus the occurrence of a P→S shunt. However, if the muscle surrounding the cog-wheel valve was killed by injection of ethanol directly into the muscle or inhibited with ACh, the cog-wheel ECG and the secondary rise in RV pressure were eliminated (Figs 2B,D, 3C). In every case where the cog-wheel valve was made non-functional by treatment with ethanol, Xylocaine or ACh, neither the secondary rise in RV pressure nor P→S shunting was seen. PA pressure during systole was

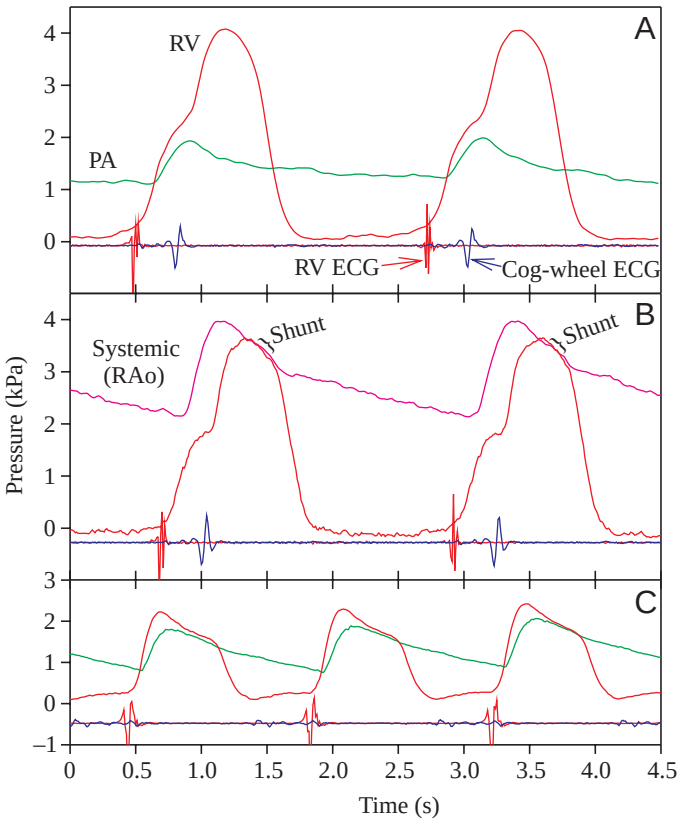

Fig. 3. Effect of cog-wheel valve contraction on pulmonary, right ventricular and systemic blood pressures in an alligator. (A) Pressure in the right ventricle (RV, red) and pulmonary artery (PA, green) and electrocardiograms (ECGs) from the centre of the right ventricle (RV ECG, red) and the cog-wheel valve muscle (cog-wheel ECG, blue) when the cog-wheel valve was functioning. (B) Pressures in the right ventricle (red) and systemic circulation (right aorta, RAo, cerise) and ECGs of the same animal with a functioning cog-wheel valve. (C) Same as A, except that the cog-wheel valve has been inactivated by injection with acetylcholine.

notably lower than RV pressure, even when the cog-wheel valve was inactivated by ACh or Xylocaine or killed by ethanol injection (see Figs 2, 3); obviously, the pulmonary outflow tract itself presented a significant resistance to blood flow.

Timing of the cog-wheel valve ECG

The delay between the RV ECG and the ECG in the cog-wheel valve muscle decreased as the electrode in the RV was moved towards the base of the heart (i.e. closer to the cog-wheel valve) (Fig. 4), suggesting that the RV ECG spread across the RV surface from the apex towards the base. The slope of the relationship between electrode separation and conduction delay is the conduction velocity across the RV and averaged 91±23 cm s^{-1} (Fig. 4). The intercept of this relationship is the nodal delay that occurred at the junction of the RV and cog-wheel muscle and averaged 248±28 ms in the three animals studied (Fig. 4). This delay was only slightly shorter than the total conduction delay measured from the centre of the RV to the cog-wheel valve muscle (267±21 ms, *N*=9), which includes both the time for the action potential to sweep over the RV and the delay associated with its passage across the RV/cog-wheel junction. Thus, approximately 90 % of the total conduction delay was due to a nodal delay at the RV/cog-wheel junction. The mean phase of the cog-wheel muscle ECG was 13.2±1.89 % of a cardiac cycle. There was a highly significant relationship between phase and heart rate (Fig. 5A) and between phase and absolute delay (Fig. 5B). There was no relationship between the absolute ECG delay and heart rate (Fig. 5A).

Because of the slow heart rates exhibited by these animals (28.6±2.2 beats min^{-1}), it was possible to confirm visually that contraction of the muscle surrounding the cog-wheel valve coincided with the cog-wheel muscle ECG. Further evidence that the cog-wheel muscle ECG coincided with valve contraction comes from the changes in RV and PA pressures that followed the cog-wheel muscle ECG (see above). We did not detect a deflection in the cog-wheel ECG that might signify termination of the action potential (equivalent to a ventricular 'T' wave). However, relaxation of the cog-wheel valve muscle preceded relaxation of the ventricle by a period great enough that it could easily be observed; the duration of the cog-wheel contraction was considerably shorter than that of the ventricle.

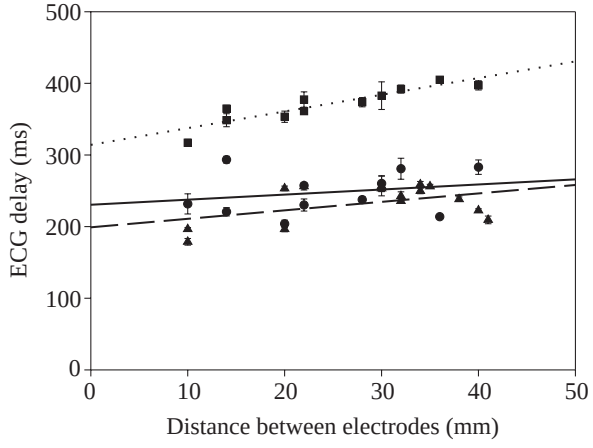

Fig. 4. Delay between the electrocardiogram (ECG) in the right ventricle and the ECG in the cog-wheel valve muscle as a function of the distance between the two recording sites. One ECG electrode was left fixed in the middle of the cog-wheel valve muscle, while the electrode in the right ventricle was moved to different locations. Results from three animals are shown. Inverse slopes give the conduction velocity in the right ventricle, and are 0.43 m s^{-1} (P<0.001, r^2=0.449), 1.41 m s^{-1} (P=0.14, r^2=0.037) and 0.85 m s^{-1} (P<0.001, r^2=0.152) from top to bottom, respectively. The intercept is the 'nodal' delay at the junction of the right ventricle and cog-wheel valve muscle. Values are means ± S.E.M.

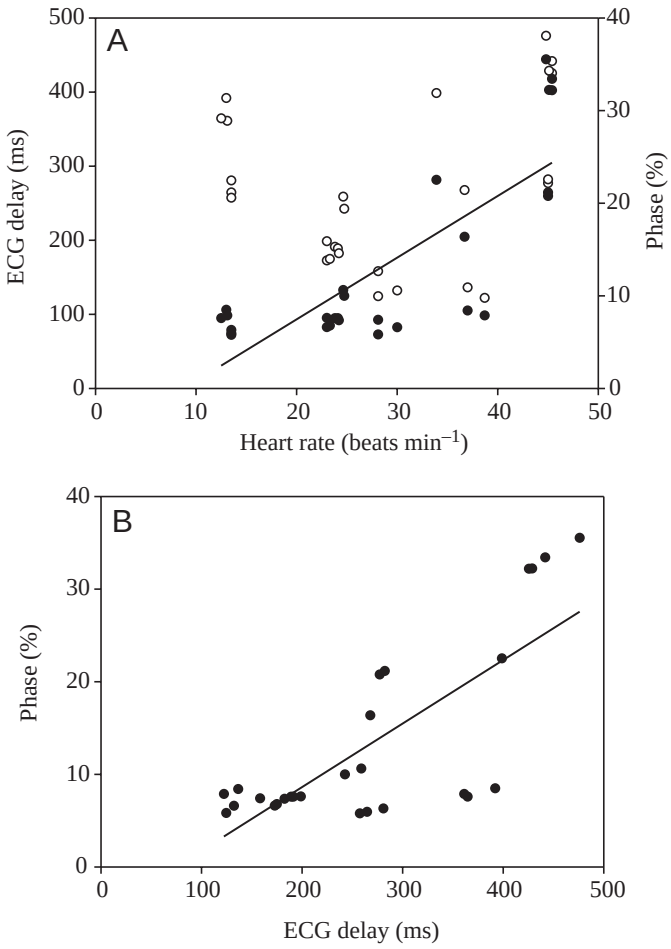

Fig. 5. (A) Relationships between the delay separating the electrocardiogram (ECG) in the middle of the right ventricle from that in the cog-wheel valve muscle and heart rate (open circles) and between the phase of the cog-wheel muscle ECG and heart rate (filled circles): delay *versus* heart rate was not significant (P=0.24); phase *versus* heart rate slope=0.67 (P<0.001, r^2=0.63). (B) Relationship between phase and the delay between the right ventricle and cog-wheel valve muscle ECGs: slope=0.069 (P<0.001, r^2=0.58).

Table 1. *Effects of left vagal stimulation, acetylcholine application and Xylocaine application on heart rate, the delay between the right ventricular and cog-wheel electrocardiograms (ECGs), the phase of the cog-wheel ECG and the integrated cog-wheel ECG*

	% of control value			
Manipulation	Heart rate	Cog-wheel ECG delay	Cog-wheel ECG phase	Cog-wheel ECG integral
Vagus (50 Hz)	56.4	78.0	44.0	92.3
Vagus (20 Hz)	40.7	74.2	30.2	88.5
Vagus (10 Hz)	27.7	76.4	21.1	79.6
ACh[1]	36.2	78.3	28.3	53.7
Vagus (10 Hz)	67.0	84.2	56.5	82.5
Xylocaine[5]	71.2	123.7	88.8	102.7
ACh[2]	65.8	62.4	40.8	85.8
ACh[3]	83.4	110.6	92.3	77.7
ACh[3]	77.2	109.4	84.4	47.6
ACh[3]	78.2	ECG abolished	ECG abolished	ECG abolished
ACh[3]	57.7	158.7	91.6	25.5
ACh[4]	85.3	138.7	118.3	88.8
Xylocaine[6]	136.1	ECG abolished	ECG abolished	ECG abolished

Values are expressed as a percentage of the control measurements that bracketed each experimental trial.

Phase is the ECG delay as a fraction of the heart beat duration. The pulse frequency during vagal stimulation is shown in parentheses (0.05 ms pulse duration, 5–7 V). Spaces separate results for individual experimental animals.

[1]0.5 ml of 2 mg ml^{-1} acetylcholine (ACh) on the right ventricle.

[2]One drop of 10 mg ml^{-1} ACh on the cog-wheel valve muscle.

[3]Two drops of 10 mg ml^{-1} ACh on the cog-wheel valve muscle.

[4]0.2 ml of 10 mg ml^{-1} ACh injected into the cog-wheel valve muscle.

[5]2 ml of 2 % lidocaine HCl (Xylocaine) injected into the cog-wheel valve muscle.

[6]Xylocaine dripped onto the cog-wheel valve muscle.

Manipulation of valve function

Conduction delay was quite constant for a given heart working at a particular heart rate. However, it changed when the heart was vagally stimulated or when ACh was applied to the cog-wheel valve muscle. In the two animals in which vagal nerve stimulation was successful, heart rate, conduction delay and cog-wheel phase all decreased substantially (Table 1). There was also a decrease in the integrated cog-wheel ECG (Table 1), signifying a weakening of cog-wheel muscle contraction. From our limited data, it appeared that the lower stimulation frequencies (10 and 20 Hz) were more effective than the high stimulation frequency (50 Hz) at eliciting these effects, although no clear pattern between vagal stimulation frequency and cog-wheel ECG delay emerged.

All four animals which had ACh applied topically to the cog-wheel valve muscle showed a decrease in heart rate and a considerable weakening of the integrated cog-wheel ECG (Table 1). The effect on the conduction delay was variable, ranging from an increase of almost 60 % to a decrease of 40 % to complete blockade of cog-wheel muscle contraction. Together with the reduction in the integrated cog-wheel ECG was a reduction in the secondary rise of pressure in the RV (Figs 2B,D, 3C). All these effects were reversible as the ACh washed out.

The effects of application of Xylocaine to the cog-wheel muscle were variable in the two instances it was attempted (Table 1). In one case, the cog-wheel muscle was inactivated and in the other case there was an increase in conduction delay but no effect on the integrated cog-wheel ECG.

Discussion

The data presented here demonstrate a synchronization between RV contraction and cog-wheel valve contraction (Figs 2–5), that the timing of these events can be altered (Table 1), that the timing accounts for the observed central pressure profiles (Figs 2, 3) and that the delay between these two events with subsequent valve contraction is likely to control P→S shunting (Fig. 3B). Further, we show that contraction of the cog-wheel valve follows RV depolarization by a defined delay (see also White, 1968), implicating a nodal conduction delay linking the RV ECG with that in the cog-wheel muscle (Figs 4, 5). From these results, it is apparent that active control over the timing of valve closure allows pulmonary input resistance and thus shunting to be controlled somewhat independently of systemic blood pressure or chronic pulmonary resistance and allows the shunt to be initiated or terminated in, perhaps, a single heart beat.

Although we believe that synchronization of RV depolarization with valve contraction is the primary mechanism that initiates/controls shunting, a number of secondary

mechanisms by which P→S shunting may be influenced have also been proposed. These include: (i) alterations in RV contractility, (ii) alterations in pulmonary resistance, including those associated with ventilation, (iii) changes in systemic vascular resistance, and (iv) changes in right ventricular end-diastolic volume (*via* a Starling effect) and, thus, changes in RV pressure development (Shelton and Jones, 1991; Axelsson and Franklin, 1997; Hicks, 1998).

More recently, Franklin and Axelsson (2000) report that shunting may be influenced through β-adrenergic control of resistance in the subpulmonary conus, which contains the cog-wheel valve. Injection of the competitive β-adrenergic antagonist sotalol into the right side of the heart increased resistance in the pulmonary outflow tract and induced shunting in isolated, perfused hearts of the estuarine crocodile *Crocodylus porosus*. The subsequent addition of a saturating concentration of adrenaline caused the shunt to be abolished. While such observations support the idea that β-adrenergic stimulation can affect the shunt, the mechanism they propose (direct, adrenergic stimulation of the cog-wheel muscle causing valve relaxation) is seemingly at odds with the normal response of cardiac muscle to adrenergic stimulation. If the cog-wheel muscle mass is of the cardiac type, which it appears to be, adrenergic stimulation would be expected to cause valve closure and thus promote P→S shunting, and removal of β-adrenergic stimulation by sotalol treatment would diminish the force of cog-wheel valve contraction and inhibit shunting; this is exactly opposite to what was observed. This leads us to the conclusion that either the cog-wheel muscle's response to adrenergic stimulation is very unusual (relaxation) or perhaps that the fibre orientation in the valve is such that contraction leads to valve opening (M. Axelsson, personal communication).

Interestingly, adrenergic stimulation of the aortic outflow tract in crocodiles leads to vasoconstriction (Axelsson and Franklin, 2001), presumably through an α-adrenergic response. If the pulmonary outflow tract behaves similarly (which we do not know at this point), then adrenergic stimulation would promote shunting. This would not be consistent with the effects of adrenergic stimulation on shunting noted by Franklin and Axelsson (2000) nor with its effects in animals; adrenergic stimulation in alligators increases systemic blood pressure (Shelton and Jones, 1991) to levels that may exceed the pressure that can be developed by the RV, and disturbing instrumented alligators increases pulmonary blood flow and always terminates shunting (D. A. S., K. G. and D. R. J., unpublished observations). However, a β-adrenergic mechanism that promoted vasodilation in the pulmonary outflow tract would be in accord with a loss of the shunt under adrenergic tone. Sustained, β-adrenergic dilation of the pulmonary outflow tract could decrease the ability of the animal to shunt by two mechanisms. First, dilation of the pulmonary outflow tract may significantly increase blood flow into the PA during early systole, leaving only a small volume of blood in the RV. This may prevent the RV from developing the pressure required to initiate a P→S shunt. Second, dilation in close proximity to the cog-wheel valve may expand the subpulmonary conus to such a degree that the cog-wheel valve could not effectively occlude the pulmonary outflow tract. It will be most interesting to learn what the effects of adrenergic stimulation on the pulmonary outflow tract are and what the anatomy of the cog-wheel valve musculature is in relation to its behaviour under adrenergic stimulation.

Cog-wheel valve contraction and shunting

In our anaesthetized alligators, there appeared to be a substantial resistance in the pulmonary outflow tract that was independent of cog-wheel valve contraction (but see Axelsson et al. (1996) for an example in crocodiles where there is very little resistance). This resistance caused a large pressure drop between the RV and PA during early systole, before the cog-wheel ECG (Figs 2A,C, 3A) (see also Shelton and Jones, 1991; Grigg and Johansen, 1987), and it did not disappear when the cog-wheel muscle was inactivated (Figs 2B,D, 3C). The catheter used to measured PA pressure was placed just distal to the cog-wheel valve, and the majority of the resistance we measured would therefore have resided within or very close to the valve. However, despite the substantial pulmonary resistance when the cog-wheel muscle was not active, RV pressures never attained levels required for shunting (Fig. 2) and, as far as we are aware, the shunt is always accompanied by a biphasic RV pressure profile showing the dramatic secondary rise in pressure associated with phasic, cog-wheel valve contraction. The resistance appears to lessen as pulmonary and RV pressures rise (Jones and Shelton, 1993; D. A. S., K. G. and D. R. J., unpublished observations), which would be consistent with the cartilaginous nodules of the cog-wheel valve being 'blown open' at higher pressures.

Inhibition of cog-wheel valve contraction, whether by ethanol, ACh, vagal stimulation or Xylocaine, caused RV pressure to track the lower PA pressure throughout systole, and there was no possibility of a shunt. Similar pressure profiles in the RV and pulmonary outflow tract, reflecting activity and inactivity of the cog-wheel valve, have also been observed during chronic recordings from the RV and PA of unanaesthetized, estuarine crocodiles (Axelsson and Franklin, 1997). Thus, while we agree with Franklin and Axelsson (2000) that shunting in crocodilians is influenced by the relative resistances in the lung *versus* systemic circulations and that the major control site of this resistance is the subpulmonary conus, it does not appear that maintained tonus in the subpulmonary conus induced by adrenergic withdrawal is adequate in itself to elicit shunting; active contraction of the valve following muscle depolarization is required. In support of this, we provide direct evidence correlating valve contraction and closure (the cog-wheel valve ECG) with the secondary rise in RV pressure that always precedes shunting (Figs 2, 3).

Cog-wheel ECG

In our study, the wave of depolarization spread across the RV from the apex towards the base with a conduction velocity of 91 cm s^{-1}. This conduction pattern is similar to that observed

by Christian and Grigg (1999) in crocodiles; however, the conduction velocity they report (65 cm s^{-1}) is much slower. Differences in temperature or species may contribute to this discrepancy; Christian and Grigg (1999) do not report the temperature used in their experiments. The cog-wheel valve muscle, located at the base of the RV, appeared to be activated by a depolarization that originated in the RV, and in each animal the two events were synchronized by a relatively consistent delay. Approximately 90 % of the delay between the RV ECG and the cog-wheel valve ECG appeared to reside at the junction between the two muscle masses (Fig. 5). These data imply that a mechanism akin to AV nodal delay exists in the alligator heart.

The existence of such a node and the physiological mechanism responsible for the delay have not previously been described in the alligator heart. Burggren (1978) noted a similar phenomenon in turtles, where a delay of 200–300 ms existed between depolarization of the ventricle and the bulbus cordis surrounding the PA. He attributed the delay to a slow conduction velocity (2 cm s^{-1} or one-fifth to one-tenth of that of the ventricle) in the transition zone between the cavum venosum of the ventricle and the bulbus cordis. When watching the contraction of the cog-wheel muscle in alligators, it appeared that the entire muscle mass contracted synchronously and, hence, it is unlikely that the delay we report was due to very slow propagation across the cog-wheel muscle mass itself. However, we did not measure conduction velocities across the small cog-wheel muscle to confirm this. White (1968) alludes to an unpublished observation of a 350 ms delay in alligators at 25 °C. In our alligators, the conduction delay averaged 248 ms at 30 °C. Pressure recordings indicate that this is long enough to allow some PA flow during early systole (a rise in PA pressure), but short enough to obstruct the PA before the full stroke volume is delivered to the lungs. Sufficient blood then remains in the RV to cause a substantial secondary rise in RV pressure during the latter half of systole and, thus, P→S shunting (Figs 2, 3).

The absolute conduction delay was not dependent on heart rate, but the phase was (Fig. 4A). Thus, changes in phase appear to be more indicative of changes in heart rate (diastolic interval) than of changes in ECG delay. It may be that the absolute delay is maintained within a relatively constant range because the timing of cog-wheel valve closure relative to the onset of RV systole would be critical in controlling shunting, rather than the phase *per se*. The significant relationship between phase and ECG delay (Fig. 4B) may simply reflect the constancy of heart rates in these animals, such that any change in delay would translate directly into a change in phase.

Changing the delay of the cog-wheel ECG or the ECG magnitude (strength of cog-wheel contraction) may be mechanisms by which the degree of shunting can be controlled. The existence of specialized fibres whose conduction velocity is under autonomic control is well established in reptilian hearts (Burggren, 1978; Christian and Grigg, 1999; and references therein). If the cog-wheel ECG occurred very late in ventricular systole, most of the RV output would be sent to the lungs, and a shunt would not occur. Alternatively, if the timing of cog-wheel valve contraction were shifted earlier in systole, more of the cardiac output could be shunted back to the systemic circulation.

Vagal stimulation in alligators resulted in a marked decrease in the absolute delay and phase of the cog-wheel ECG (Table 1). Further, Malvin et al. (1995) found that efferent vagal stimulation in alligators promoted pulmonary vasoconstriction. Both these responses would favour P→S shunting. However, the integrated cog-wheel ECG was decreased under vagal stimulation (Table 1), which presumably reflects an inhibition of valve function and a decreased capacity to shunt. In turtle hearts, peripheral stimulation of the cut vagus or application of ACh causes the ventricular depolarization pattern to shift transiently from that seen during apnoea to that observed during breathing, and the absolute magnitude of the conduction delay is longer during breathing than during apnoea (Burggren, 1978). Both these changes would favour blood flow to the lungs (inhibit shunting), the latter by the mechanism we propose. In our experiments, the effect of ACh on the conduction delay was variable (Table 1) and at present does not support a role for a direct effect of ACh on the timing of valve closure and shunting. However ACh, like vagal stimulation, did appear to weaken the cog-wheel ECG, and this may inhibit shunting. Shelton and Jones (1991) did not see any effect of ACh administration on the relative timing of events in the left and right ventricles. In contrast, White (1970) noted that atropine injection reversed both the diving-induced bradycardia and the large RV–PA pressure gradient in alligators, suggesting that the shunt may also be cholinergically influenced.

Concluding remarks

The cog-wheel valve in alligators is surrounded by a muscle mass that is somewhat isolated from the RV. This muscle produces a distinct ECG signal that is temporally separated by approximately 250 ms from the RV ECG measured at the base of the heart. The cog-wheel ECG signals the onset of valve closure and obstruction of the pulmonary outflow tract, resulting in a phasic, secondary rise in RV pressure and a fall in PA pressure, and sets up the haemodynamic conditions required for P→S shunting. The extent of the delay could have a major influence on RV pressure and the degree of shunting. Vagal and cholinergic stimulation had significant but varied effects on the cog-wheel ECG. Both appeared to weaken cog-wheel valve contraction, which may inhibit shunting. Vagal stimulation decreased the delay between the RV and cog-wheel ECGs, which could promote shunting. Although these latter results do not provide a clear picture of how autonomic nervous tone would control the extent of P→S shunting, they do, in combination with the results of Franklin and Axelsson (2000) and Axelsson and Franklin (2001), provide strong evidence that nervous and/or humoral mechanisms acting on the subpulmonary conus and valve can markedly influence the magnitude of P→S shunting in crocodilians.

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