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SHORT COMMUNICATION

Contribution of active atrial contraction to cardiac output in anesthetized American alligators (*Alligator mississippiensis*)

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ABSTRACT

Ventricular filling may occur directly from the venous circulation during early diastole or via atrial contraction in late diastole. The contribution of atrial contraction to ventricular filling is typically small in mammals (10–40%), but has been suggested to predominate in reptiles. We investigated the importance of atrial contraction in filling of the ventricle in American alligators (*Alligator mississippiensis*) by bypassing both atria (with the use of ligatures to prevent atrial filling) and measuring the resultant effects on cardiac output in anesthetized animals. Atrial ligation had no significant effects on total systemic blood flow before or after adrenaline injection. Unexpectedly, pulmonary flow was increased following atrial ligation prior to adrenaline treatment, but was unaffected after it. These findings suggest that the atria are non-essential (i.e. redundant) for ventricular filling in alligators, at least under anesthesia, but may serve as important volume reservoirs.

KEY WORDS: Cardiac filling, Crocodilian, Reptile, Adrenaline

INTRODUCTION

Ventricular filling, which establishes cardiac stroke volume (V_S) and is hence a key determinant of cardiac output ($\dot{Q}$), may occur directly from the venous circulation during early diastole, or via active atrial contraction in late diastole (the 'atrial kick'). Historically, the relative contribution of atrial contraction to ventricular filling was addressed in De Motu Cordis, where William Harvey stated that 'blood enters ventricles… by being thrown into them by the pulses of the auricles [atria]' (Harvey, 1628). This declaration was primarily based on visual observations of ectothermic vertebrates, including fishes, frogs and snakes (Henderson, 1906). Later studies on mammalian (typically canine) heart preparations suggested that the atrial contribution is considerably less important. Indeed, Henderson (1906) concluded that 'contraction of the auricles [atria] increases the ventricular volume at the most to the extent of a few drops'. Subsequent work, using more refined techniques (Straub, 1910), demonstrated the atrial kick may be more pronounced and contribute to 18–60% of normal ventricular filling (Gesell, 1916; Wiggers and Katz, 1922). Mitchell et al. (1965) demonstrated that the atrial contribution to ventricular filling depends on heart rate (f_H) in dogs, such that the atria are responsible for 20% of ventricular filling at low f_H and 37% at higher f_H (Mitchell et al., 1965); a similar phenomenon also applies to humans (e.g. Samet et al., 1964, 1965). The contemporary view on the mammalian heart maintains that the atrial contribution to ventricular filling is approximately 10% at low f_H, but may increase to 40% when passive filling time is shortened at high f_H (Woerlee, 2012). In birds, which, like mammals, have a fully divided ventricle and high f_H to support the metabolic demands of endothermy, the role of atria in ventricular filling has not been directly investigated, although it has been suggested that, because many species lack sinoatrial valves, atrial contraction plays a minimal role in filling the ventricles (Smith et al., 2000).

Despite Harvey's initial advancements, the role of atrial contraction in ectothermic vertebrate hearts remains considerably less understood. Given the relatively short ventricular diastole in reptiles, which coincides with atrial contraction, Johansen (1959) and Johansen and Burggren (1984) concluded that atrial contraction is the primary source of ventricular filling in snakes and varanid lizards. However, more recent combinations of electrocardiograms with echocardiography in python hearts revealed open atrioventricular valves during ventricular relaxation, prior to atrial contraction, indicating the possibility of venous filling of the ventricle before the atrial kick (Jensen et al., 2010). This is consistent with earlier experiments on excised turtle ventricles, which, in the absence of atria, had to fill via suction (i.e. elastic recoil) during ventricular diastole (Katz, 1930; Kraner, 1959).

In the present study, we directly investigated the importance of atrial contraction for ventricular filling by ligating the majority of both left and right atria, thus forcing blood to bypass the atria into the ventricles, in anesthetized American alligators (*Alligator mississippiensis* Daudin 1802). We hypothesized that if atrial contraction is critical for ventricular filling, atrial ligation would decrease V_S and $\dot{Q}$. Given that the atrial contribution varies with f_H and $\dot{Q}$ in mammals, we investigated the effects of adrenaline before and after atrial ligation. Further, because atrial ligation required opening the pericardium, and this has previously been shown to increase $\dot{Q}$ in perfused crocodile hearts (Axelsson and Franklin, 1995), we also evaluated the effects of adrenaline before and after opening the pericardium.

MATERIALS AND METHODS

Experimental animals

We studied six female American alligators (*A. mississippiensis*; mean±s.d. body mass=3.08±0.61 kg; age: 3 years) that were hatched from eggs obtained from the Rockefeller Wildlife Refuge in Grand Chenier (Louisiana, USA). The alligator eggs were incubated under hypoxia (10% O_2), as previously described (Eme et al., 2011), which represents an environmentally relevant condition for crocodilian nests (Lutz and Dunbar-Cooper, 1984). After hatching, the juvenile animals were maintained under normoxic conditions (21% O_2) in freshwater in 0.7×2×0.7 m fiberglass pens after hatching. The animals were fed commercial alligator food twice weekly and maintained under a 12 h:12 h light: dark cycle. The experiments were approved by the University of North Texas animal ethics committee IACUC (no. 17-001).

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Surgical procedures

Anesthesia was induced by placing the head of the alligator in a Ziploc bag containing cotton gauze soaked in isofluorane (Isothesia, Henry Schein Animal Health, Dublin, OH, USA). The animal's trachea was then intubated with Tygon tubing and ventilated (6 breaths min^{-1}; 15 ml kg^{-1}) using a mechanical ventilator (model 665, Harvard Apparatus, Holliston, MA, USA). Isofluorane was maintained at 1.5–2%, during instrumentation, using an isofluorane vaporizer (Highland Medical Equipment, Temecula, CA, USA). Body temperature was maintained at 29±0.5°C with a heating lamp connected to a Thermistemp temperature controller (model 71A, YSI, Yellow Springs, OH, USA). The major arteries were blunt dissected, allowing the placement of Transonic flow probes (Transonic Systems, Ithaca, NY, USA) around the left pulmonary artery ($\dot{Q}_{Lpul}$), the left aortic arch ($\dot{Q}_{LAo}$), the common carotid artery $\dot{Q}_{Car}$ and both the right aortic arch and the right subclavian artery ($\dot{Q}_{RAo+Sc}$). The use of a single probe to measure flow in two parallel vessels has been previously validated in turtles (Wang and Hicks, 1996; Wearing et al., 2017). The right precaval vein (Reese, 1915) was occlusively cannulated with a PE-50 catheter to allow adrenaline injections (see below). The flow probes were connected to T402 and T403 blood flow meters (Transonic Systems). The outputs from the transonic meters were recorded with a PowerLab® 16/35 connected to a computer running LabChart Pro® software (v 8.2, ADInstruments, Colorado Springs, CO, USA), and data were recorded at 100 Hz.

Experimental protocol

After instrumentation, isofluorane was reduced to 1%, and 30 min was allowed for cardiovascular variables to stabilize before adrenaline (5 nmol kg^{-1}; 0.1 ml kg^{-1} bolus) was injected into the venous cannula. Once all variables returned to baseline (20 min), the pericardium was opened with an incision that exposed the ventricles and atria. After this procedure, an additional stabilization period (20 min) was allowed before the adrenaline injection was repeated. Finally, once baseline was re-established (20 min), both atria were ligated with 4-0 silk. Filling of the vast majority (>80%) of the atria was prevented by tying the silk near the base of each atrium, without preventing blood flow directly from the sinus venosus through the atrio-ventricular valves. The adrenaline injection was then repeated a final time.

Calculations and statistical analysis

All blood flows were corrected for temperature using previously generated calibration curves for each individual flow probe at 30°C, which accounts for the temperature sensitivity of the probes that were factory calibrated at 37°C. Total systemic flow ($\dot{Q}_{sys}$) was calculated from the sum of $\dot{Q}_{RAo+Sc}$, $\dot{Q}_{Car}$ and $\dot{Q}_{LAo}$. Total pulmonary flow ($\dot{Q}_{pul}$) was estimated by doubling $\dot{Q}_{Lpul}$, presuming blood flow in each pulmonary artery is equal. Systemic stroke volume (V_{Ssys}) and pulmonary stroke volume (V_{Spul}) were calculated by dividing $\dot{Q}_{sys}$ and $\dot{Q}_{pul}$, respectively, by f_H.

The mean values for a 5 min sample period represented the pre-treatment and peak post-treatment (injection of adrenaline, pericardium cut, atrial ligation) response. All measurements were analyzed for differences with a repeated-measures ANOVA (GraphPad Prism v7.0a, GraphPad Software, La Jolla, CA, USA). Sidak *post hoc* tests were used for pairwise comparisons between pre-treatment and post-treatment values. Data are presented as means±s.e.m., with statistical significance designated when $P<0.05$.

RESULTS AND DISCUSSION

Prior to physical manipulations (cutting the pericardium and ligating the atria), adrenaline increased f_H from approximately 20 to 30 beats min^{-1} (Table 1), with an associated rise in $\dot{Q}_{sys}$ (70% increase) and $\dot{Q}_{pul}$ (>200% increase) (Fig. 1A,B). The largest effect on the individual systemic flows was an approximate doubling of $\dot{Q}_{RAo+Sc}$, whilst $\dot{Q}_{Car}$ and $\dot{Q}_{LAo}$ changed much less (Table 1). V_{Ssys} (Fig. 1C) did not significantly change following adrenaline; however, V_{Spul} approximately doubled (Fig. 1D). In perfused crocodile (*Crocodylus porosus*) hearts, Axelsson and Franklin (1995) also reported an adrenaline-induced tachycardia (from 33 to 39 beats min^{-1}), but because V_S decreased there was no change in $\dot{Q}$. In their study, the decrease in filling time presumably reduced cardiac preload (e.g. Altimiras and Axelsson, 2004), which, in the absence of pronounced inotropic effects (see Joyce et al., 2017a), was likely responsible for the decrease in V_S. However, in the intact circulatory system of the anesthetized alligators, adrenaline also exerts direct effects on vascular capacitance. Although we did not measure it in the present study, it is well established that adrenaline increases central venous pressure in other reptiles (rattlesnakes: Skals et al., 2005; turtles: Joyce et al., 2017b), which would compensate for the reduction in V_S via the Frank–Starling mechanism in spite of the tachycardia.

$\dot{Q}_{pul}$ may have increased more than $\dot{Q}_{sys}$ because of adrenaline's β-adrenoceptor-dependent action on the cog-teeth valves, which are unique to crocodilians and are able to restrict $\dot{Q}_{pul}$ (Franklin and Axelsson, 2000). $\dot{Q}_{pul}$ may also have been favored owing to an increase in systemic vascular resistance (e.g. Jensen et al., 2015), which is the main regulator of shunt patterns in non-crocodilian reptiles (Hicks and Wang, 2012). It is also plausible that pulmonary vascular resistance was decreased by adrenaline, although this is seldom the case in non-crocodilian reptiles (turtles: Overgaard et al., 2002; rattlesnakes: Galli et al., 2007).

Opening the pericardium had no significant effect on any of the measured cardiovascular variables before or after adrenaline treatment. This contrasts with the finding in perfused crocodile hearts that opening the pericardium increases $\dot{Q}$ and V_S, although this was only previously investigated during maximum adrenergic stimulation (Axelsson and Franklin, 1995). The comparisons in perfused hearts were only made at equivalent filling pressures, and it is possible that opening the pericardium altered (i.e. decreased) the filling pressure (e.g. Spotnitz and Kaiser, 1971), thus normalizing $\dot{Q}$ in the anesthetized animals. It is also plausible that any of the discrepancies between our data and those of Axelsson and Franklin (1995) on the effects of adrenaline or opening the pericardium reflect, in part, differences between estuarine crocodiles (*C. porosus*) and American alligators (*A. mississipiensis*), although their cardiovascular dynamics and morphology appear to be similar (Axelsson and Franklin, 1997).

Atrial ligation had no significant effect on $\dot{Q}_{sys}$ before or after adrenaline treatment (Fig. 1A), thus we must reject our original hypothesis that atrial filling and contraction is an essential contributor to ventricular filling in alligators. This contradicts the previous dogma in reptiles (Johansen, 1959; Johansen and Burggren, 1984), although these earlier studies failed to directly test the assumption and only considered non-crocodilian reptiles (snakes and varanid lizards), which possess undivided ventricles and markedly different cardiac anatomy to crocodiles (e.g. Hicks and Wang, 2012). It was particularly surprising that the negligible role for atrial contraction was apparent even when f_H was increased by adrenaline. In this circumstance, the shortened filling time was probably counteracted by the positive lusitropic effect of adrenaline that promotes ventricular suction (Kraner, 1959).

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Table 1. The effects of adrenaline (5 nmol kg^{-1} bolus injection) on heart rate (f_H) and individual systemic blood flows [combined right aortic arch and right subclavian artery ($\dot{Q}_{RAo+Sc}$), common carotid artery ($\dot{Q}_{Car}$) and left aortic arch ($\dot{Q}_{LAo}$)] in anesthetized American alligators

	f_H (beats min^{-1})	$\dot{Q}_{RAo+Sc}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{Car}$ (ml min^{-1} kg^{-1})	$\dot{Q}_{LAo}$ (ml min^{-1} kg^{-1})
Control				
Untreated	20.5±1.6	4.8±1.3	7.1±0.7	5.6±0.7
Adrenaline	30.6±1.6*	10.7±1.6*	10.7±1.2*	8.5±1.2*
Open pericardium				
Untreated	21.1±1.6	5.5±1.5	6.5±0.7	6.3±0.8
Adrenaline	30.0±1.6*	11.7±1.3*	9.1±0.7	8.5±1.3*
Atria ligated				
Untreated	22.9±2.4‡	5.1±1.1	6.2±0.7	7.7±1.4‡
Adrenaline	31.5±2.4*	10.9±0.8*	10.9±0.8*	8.7±1.7

The effects of adrenaline were investigated in animals with an intact heart (control), after opening the pericardium (open pericardium) and after ligating both atria to prevent atrial filling during diastole (atria ligated). Asterisks indicate a significant effect of adrenaline within a condition; double daggers indicate a significant difference between atrial-ligated and control states. *N*=6; values are presented as means±s.e.m.

A further unexpected finding was that atrial ligation increased $\dot{Q}_{pul}$, prior to adrenaline treatment, by approximately 50% (Fig. 1B) and also modestly increased $\dot{Q}_{LAo}$ (Table 1). f_H exhibited a small increase after atrial ligation (~2.5 beats min^{-1}; Table 1), but the increase in $\dot{Q}_{pul}$ was primarily due to an increase in V_{Spul} (Fig. 1D). Reptilian atria, especially the right atrium (Jensen et al., 2014), are large and may store a considerable volume of blood. When the atria were ligated, this blood was mobilized into the circulation, which may have been responsible for the increase in $\dot{Q}_{pul}$. Consistent with this hypothesis, $\dot{Q}_{pul}$ was similar in non-ligated and ligated states after adrenaline treatment, which may have maximized (i.e. saturated) $\dot{Q}$ by decreasing vascular capacitance in both circumstances. Indeed, the role of atria as blood reservoirs was originally proposed by Henderson (1906) when he described their limited role in ventricular filling.

The maximum f_H following adrenaline treatment (~30 beats min^{-1}) was substantially lower than maximum f_H recorded in alligators during terrestrial treadmill exercise (~60 beats min^{-1}; Munns et al., 2005), immediately following disturbance (~50 beats min^{-1}; Axelsson et al., 2007) and during swimming (~45 beats min^{-1}; W.J., R.M.E., T.W. and D.A.C. II, unpublished) at similar temperatures (~30°C). This is most likely

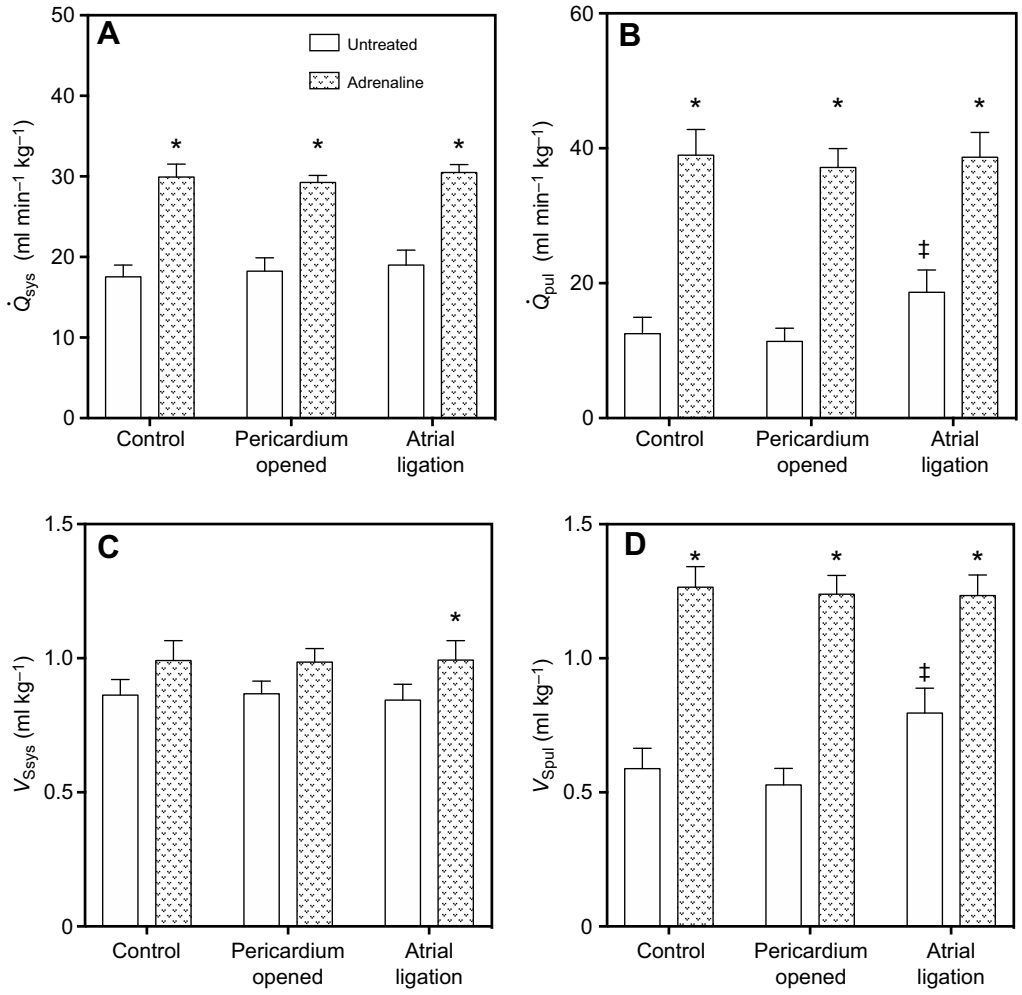

Fig. 1. The effects of adrenaline (5 nmol kg^{-1} bolus injection) on blood flows and stroke volumes in anesthetized American alligators with an intact heart (control), after opening the pericardium (open pericardium) and after ligating both atria to prevent atrial filling during diastole (atria ligated). (A) Total systemic flow ($\dot{Q}_{sys}$), (B) total pulmonary flow ($\dot{Q}_{pul}$), (C) systemic stroke volume (V_{Ssys}) and (D) pulmonary stroke volume (V_{Spul}). Asterisks indicate a significant effect of adrenaline within a condition; double daggers indicate a significant difference between atrial-ligated and control states. *N*=6; values are presented as means± s.e.m.

attributable to the direct negative chronotropic effect of isofluorane on the cardiac pacemaker (Stoyek et al., 2017). As the atrial contribution to ventricular filling increases considerably at high f_H in mammals (Mitchell et al., 1965; Woerlee, 2012), it is certainly possible that we underestimated the potential atrial contribution at maximum $\dot{Q}$ (e.g. during activity) in alligators. However, the invasive intervention of atrial ligation is not immediately suitable for recovered animals.

Whilst our data suggest that atrial function is not obligatory in anesthetized alligators, we cannot exclude a facilitative role of atrial contraction in filling of the ventricle in recovered animals. Indeed, we cannot refute Harvey's (1628) classical observation that the atria superficially appear to play a major role in filling the reptilian ventricle; this is also obvious from our own visual observations. Following ligation, passive suction of the ventricle may be enhanced and compensate filling to maintain $\dot{Q}$. Conversely, there is a medical report of a human patient with a 'stuck' mitral valve, meaning that passive filling of the ventricle was prevented during early diastole and could only be achieved by atrial contraction to forced the valve open (Neuman et al., 2011). Thus, there appears to be a substantial redundancy between direct and atrial-assisted filling of the ventricle in both crocodilian and mammalian hearts.

It has been suggested that the reduced reliance on atrial function is a key innovation in the evolution of the endothermic heart in birds and mammals (Burggren et al., 2014). Our finding that the atria play a non-essential role in ventricular filling in alligators, at least in the anesthetized state, questions this hypothesis and suggests that a limited role for atrial-assisted filling on the ventricle may not have been linked with the evolution of endothermy. As alligators are the closest living relatives to birds, in which it is also thought that atrial filling of the ventricle is minor (Smith et al., 2000), we propose that this state may have been ancestral in archosaurs. Alligators do, nonetheless, possess a four-chambered heart (like birds and mammals), and it has been suggested that crocodiles evolved from endothermic ancestors (Seymour et al., 2004), so may not be representative of reptiles as a whole. Future studies in other species (especially non-crocodilian reptiles and birds), ideally using other interventions in recovered animals, may therefore be key to resolving the importance of the 'atrial kick' across the vertebrate phylogeny.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: W.J., T.W., D.A.C.; Methodology: W.J., J.C., D.A.C.; Formal analysis: W.J., D.A.C.; Investigation: J.C.; Resources: J.C., R.M.E., T.W., D.A.C.; Data curation: J.C., T.W., D.A.C.; Writing - original draft: W.J., T.W., D.A.C.; Writing - review & editing: R.M.E., T.W., D.A.C.; Supervision: T.W., D.A.C.; Project administration: D.A.C.

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