

SHORT COMMUNICATION

Autoregulation of cardiac output is overcome by adrenergic stimulation in the anaconda heart

William Joyce^{1,*}, Michael Axelsson² and Tobias Wang¹

ABSTRACT

Most vertebrates increase cardiac output during activity by elevating heart rate with relatively stable stroke volume. However, several studies have demonstrated ‘intrinsic autoregulation’ of cardiac output where artificially increased heart rate is associated with decreased stroke volume, leaving cardiac output unchanged. We explored the capacity of noradrenaline to overcome autoregulation in the anaconda heart. Electrically pacing *in situ* perfused hearts from the intrinsic heart rate to the maximum attainable resulted in a proportional decrease in stroke volume. However, noradrenaline, which increased heart rate to the same frequency as pacing, maintained stroke volume and thus increased cardiac output. In atrial and ventricular preparations, noradrenaline significantly increased the force of contraction and contraction kinetics. Thus, the increased contractility associated with adrenergic stimulation ameliorates filling limitations at high heart rates. Although heart rate appears the primary regulated variable during activity, this may only be achieved with compensatory amendments in myocardial contractility provided by adrenergic stimulation.

KEY WORDS: Cardiovascular, Heart rate, Stroke volume, Noradrenaline

INTRODUCTION

Cardiac output, the product of heart rate and stroke volume, delivers oxygen to respiring cells and must be adjusted as metabolic requirements change. In most vertebrates, with the exception of some fish (Farrell, 1991), periods of heightened activity are accompanied by increased heart rate, whilst stroke volume changes only modestly (Bevegård and Shepherd, 1967; Secor et al., 2000; Sandblom et al., 2005).

Whilst ‘frequency modulation’ of cardiac output dominates amongst vertebrates, changes in heart rate have ensuing effects on cardiac filling and contractility. It was first noted by Markwalder and Starling (1914) in dog heart–lung preparations that, under unchanged filling conditions, decreased heart rate is associated with a proportional elevation of stroke volume, such that cardiac output is unchanged. Cardiac output ‘autoregulation’ was later confirmed *in vivo* (Noble et al., 1966), and extended to species as diverse as rainbow trout (Altimiras and Axelsson, 2004) and humans (Munch et al., 2014). The phenomenon is primarily attributed to reduced cardiac filling time and pressure (preload pressure) at high

contraction frequencies (Markwalder and Starling, 1914; Altimiras and Axelsson, 2004). Heart rate is further entwined with myocardial contractility through the ‘force–frequency’ effect, which describes the rate-dependent change in myocardial force production that may be negative, positive or flat, and varies between species (Shiels et al., 2002; Galli et al., 2006).

In ectotherms, previous focus has centred on the importance of regulation of the venous system to adjust stroke volume during tachycardia (e.g. Sandblom et al., 2005; Skals et al., 2005, 2006; Enok et al., 2016). By mobilizing blood from the veins, preload pressure may be maintained, or even increased, despite the increased heart rate during activity (Sandblom et al., 2005; Skals et al., 2006). This ‘venocentric’ approach, however, overlooks the potential for regulation of the heart itself. Beta-adrenergic stimulation of the heart, which typically increases with exercise (e.g. Wang et al., 2001), may overcome the limitations on stroke volume by increasing myocardial contractility (Farrell et al., 1989; Altimiras and Axelsson, 2004). However, Axelsson and Franklin (1995) observed an autoregulatory response to adrenaline in *in situ* perfused crocodile hearts: heart rate increased, but stroke volume decreased. In the present study, we assess the generality of this observation and investigate the capacity of adrenergic stimulation to counter autoregulation of cardiac output in the yellow anaconda (*Eunectes notaeus* Cope 1862) heart. The anaconda heart is typical of squamate reptiles (Jensen et al., 2014) and was therefore chosen as a representative species for this taxon. Further, the *in situ* perfused heart preparation recently developed for this species (Joyce et al., 2016) allowed precise and independent manipulation of heart rate, preload pressure, afterload and adrenergic stimulation. The *in situ* studies were complemented with an *in vitro* investigation to characterize the force–frequency effect and sensitivity to noradrenaline in atrial and ventricular myocardium.

MATERIALS AND METHODS

Experimental animals

Twelve male yellow anacondas [0.33±0.05 kg (mean±s.d.)] were obtained commercially and maintained within the Aarhus University animal care facilities. The snakes were kept in individual vivaria at 28°C with access to water and fasted for at least 2 weeks prior to the study. Prior to the *in vitro* and *in situ* protocols, the animals were anaesthetized with isoflurane and decapitated. All experiments were conducted in accordance with Danish animal care regulations.

Composition of the Ringer’s solution

The *in vitro* and *in situ* protocols employed identical Ringer’s solution, composed of (in mmol l⁻¹): NaCl (95), NaHCO₃ (30), NaH₂PO₄ (1), KCl (2.5), MgSO₄ (1), CaCl₂ (2) and glucose (5), bubbled with 2% CO₂, 50% O₂ and 48% N₂ (pH 7.7). All experiments were conducted at 30°C.

¹Department of Zoophysiology, Aarhus University, Aarhus C 8000, Denmark.

²Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg 405 30, Sweden.

*Author for correspondence (william.joyce@bios.au.dk)

 W.J., 0000-0002-3782-1641

***In situ* preparations**

This experiment used seven snakes (body mass: 0.25–0.4 kg). The *in situ* perfused preparations were instrumented as described previously (Joyce et al., 2016). The posterior caval vein was cannulated with a 16 gauge stainless steel cannula to deliver perfusate to the right atrium, the left atrium was perfused by a 19 gauge stainless steel cannula in the pulmonary vein, and the remaining veins were ligated with 4/0 surgical silk. The common pulmonary artery, left aortic arch and right aortic arch were cannulated with polyethylene cannulae (outer diameter: 2 mm) immediately cranial to the heart, allowing the pericardium to remain intact. Following instrumentation, the preparation was transferred to an organ bath containing 0.9% NaCl and perfusion with Ringer's solution was resumed.

The double-bored cannulae (see Franklin and Axelsson, 1994) allowed continuous measurements of pressure at the tip of insertion. Pressure cannulae (PE-50) were connected to pressure transducers (PX600; Baxter Edwards, Irvine, CA, USA) that were calibrated against a static water column. Arterial flows were measured by ultrasonic flow-through probes (4NRB; Transonic System, NY, USA) placed in the outflow lines and connected to a Transonic T206 flow meter. Signals from the pressure transducers and flow meter were recorded with a Biopac MP100 data acquisition (Biopac Systems, Goleta, CA, USA) at 100 Hz. Heart rate was derived from the pulsatile flow signals.

The preparations were allowed at least 20 min to stabilize at intermediate preload pressure (0.2–0.4 kPa) and an afterload of 5 kPa. Thereafter, three 'Starling trials' were conducted, where preload pressure was increased in steps of approximately 0.05 kPa. The first trial was carried out at the intrinsic heart rate. For the second trial, two silver electrodes were placed on either side of the right atrium for electrical pacing of the heart using a Grass SD9 stimulator (Quincy, MA, USA) to attain a heart rate of 50 beats min^{-1} (0.83 Hz); pilot experiments on sino-atrial preparations revealed this to be the maximal heart rate attainable with adrenergic stimulation. The third trial was conducted in the presence of a saturating dose (10 $\mu\text{mol l}^{-1}$) of noradrenaline. During the second trial, pacing failed for one preparation, which was therefore excluded from the analysis.

Prior to the addition of noradrenaline, but after the paced Starling trial, we conducted an experiment under constant filling conditions (i.e. the height of the pressure column filling the heart was not changed during the trial). Preload pressure was adjusted to attain a cardiac output of approximately 35 $\text{ml min}^{-1} \text{kg}^{-1}$ and a stroke volume of 1 ml kg^{-1} in the absence of pacing. Thereafter, pacing was resumed, whilst cardiac output and preload pressure were permitted to change. Pacing lasted until flows and preload pressure stabilized (<2 min). Perfusion with noradrenaline then commenced and the intrinsic change in preload pressure, heart rate and cardiac output were followed until stabilization (<10 min).

The anaconda heart operates as a single pressure pump (Joyce et al., 2016), thus cardiac output was calculated as the sum of left aortic flow, right aortic flow and pulmonary flow and normalized to body mass (kg). Stroke volume was calculated by dividing total flow by heart rate. As right and left atrial filling pressures were controlled in tandem, preload pressure was defined as the average of right and left atrial filling pressures (kPa) (Farrell et al., 1994). For each individual preparation, flow and stroke volume were fitted to preload pressure using a third-order polynomial function. The polynomial equations from each animal were then combined to generate data at set points (0.05 kPa intervals) to produce composite

graphs consisting of means of all animals (Franklin and Axelsson, 1994; Wang et al., 2002).

***In vitro* preparations**

This experiment used five animals (body mass: 0.26–0.38 kg). The heart was removed and transferred to Ringer's solution and dissected to provide an atrial strip and a ventricular strip preparation (e.g. Joyce et al., 2014). The preparations were tied at each end with surgical silk before one end was tied to a metal rod that was attached to a force transducer (Statham UC 2, Oxnard, CA, USA). The other end was tied to one of two silver electrodes, and each preparation was immersed in 50 ml of Ringer's solution contained within a water-jacketed organ bath. After 15 min, electrical stimulation (Grass SD9 stimulators) commenced at 12 beats min^{-1} with 5 ms pulses at a voltage double that required to elicit contraction. After 15 min, the preparations were stretched with a micrometer screw until the maximum force of contraction was attained. The preparations were then allowed 30 min to stabilize.

Each preparation underwent a force–frequency trial from 12 to 60 beats min^{-1} . Each frequency was maintained until contractions stabilized. Thereafter, a saturating dose of noradrenaline (10 $\mu\text{mol l}^{-1}$) was added to the Ringer's solution and the force–frequency trial was repeated once force had stabilized (<10 min). Upon commencing the experiment, the strips were measured and weighed, allowing cross-sectional area to be estimated assuming a density of 1.0 mg mm^{-3} . At each frequency in the presence and absence of noradrenaline, twitch force, time to peak force and time to 50% relaxation were recorded, allowing the rate of contraction and rate of 50% relaxation to be calculated.

Statistical analysis

In the *in situ* perfused heart, the effect of pacing and noradrenaline on heart rate, preload pressure, stroke volume and cardiac output were analyzed with a repeated-measures one-way ANOVA. For the Starling trials, a repeated-measures two-way ANOVA was used to determine significant effects of pacing or noradrenaline on cardiac output and stroke volume at different preload pressures. In the *in vitro* experiments, the effects of noradrenaline and stimulation frequency were assessed with a repeated-measures two-way ANOVA. Differences were considered statistically significant when $P < 0.05$. Data are presented as means \pm s.e.m.

RESULTS AND DISCUSSION

To disentangle the interdependent effects of heart rate, contractility and cardiac filling, we explored cardiac performance in the anaconda (*E. notaeus*) using *in vitro* and *in situ* perfused cardiac preparations. We specifically sought to quantify the extent to which adrenergic stimulation can ameliorate cardiac filling limitations at elevated heart rates.

Right atrial pacing raised heart rate (Fig. 1A), which decreased preload pressure (Fig. 1B) and stroke volume (Fig. 1C), resulting in unchanged cardiac output (Fig. 1D). This frequency-independent 'autoregulation' is similar to that of mammals and fish; thus, the reciprocal relationship between heart rate and stroke volume appears to be a common feature of the vertebrate heart (Markwalder and Starling, 1914; Altimiras and Axelsson, 2004). At equivalent preload pressures in the Starling trials, paced hearts generated higher cardiac output than control hearts (Fig. 2A), although this was only statistically resolvable at the higher preload pressures.

The decreased stroke volume during cardiac pacing may be attributed to reduced time for ventricular filling [decreased end diastolic volume (EDV)] or compromised contractility [increased

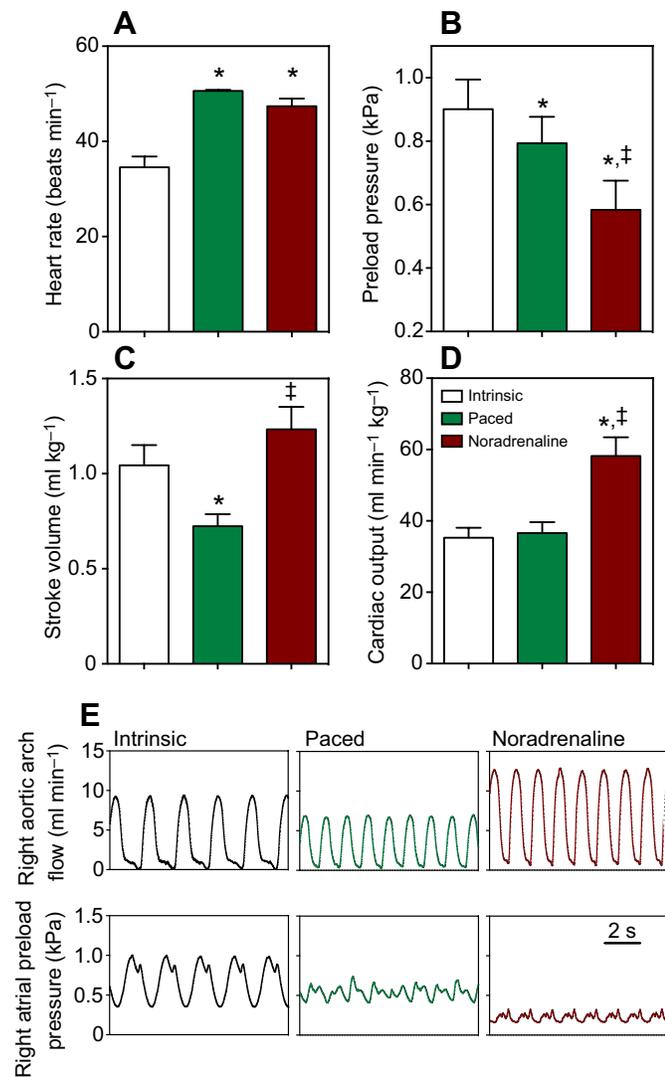


Fig. 1. Cardiac performance during control conditions and at elevated heart rates achieved by electrical pacing and adrenergic stimulation in perfused anaconda hearts. The effects of right atrial pacing (0.83 Hz) and noradrenaline ($10 \mu\text{mol l}^{-1}$) on (A) heart rate, (B) preload pressure, (C) stroke volume and (D) cardiac output in *in situ* perfused anaconda hearts under unchanged filling conditions. Asterisks indicate a significant difference from intrinsic conditions; double daggers indicate a significant difference between paced and noradrenaline treatments (repeated-measures one-way ANOVA). Values are means \pm s.e.m. ($N=7$). (E) An original trace depicting the effect of right atrial pacing and noradrenaline on right aortic arch flow and right atrial preload pressure from a 0.311 kg anaconda.

end systolic volume (ESV)]. Because ventricular twitch force was unaffected by stimulation frequency *in vitro* (Fig. 3A), contractility *per se* does not appear to be the primary limitation. Instead, the reduced preload pressure at elevated heart rate suggests that EDV is diminished. Given this finding, we established the effect of pacing across a range of preload pressures by constructing Starling curves at both intrinsic and elevated heart rates (Fig. 2). The paced hearts generated a higher cardiac output, however stroke volume remained consistently lower for a given preload pressure, suggesting that the decreased preload pressure is not solely accountable for the decreased stroke volume at higher frequencies. The temporal factor is probably important; at higher heart rates, decreased diastolic filling time restricts perfusate from entering the ventricle,

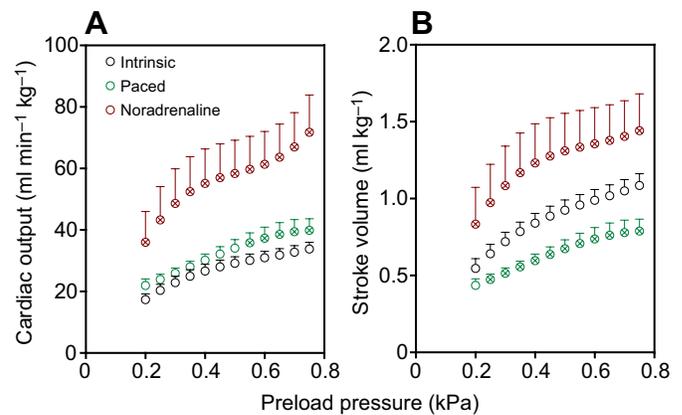


Fig. 2. The effects of electrical pacing and adrenergic stimulation on cardiac performance over a range of preload pressures in perfused anaconda hearts. The effect of right atrial pacing (0.83 Hz) and noradrenaline ($10 \mu\text{mol l}^{-1}$) on (A) cardiac output and (B) stroke volume during a Starling trial. Heart rates during the Starling trials were the same as depicted in Fig. 1A for each condition. Crossed symbols indicate a significant difference from preparations during intrinsic (untreated) conditions (repeated-measures two-way ANOVA). Values are means \pm s.e.m. ($N=6$).

regardless of the filling pressure. Further, the atrial contribution to ventricular filling, which is particularly important in ectotherm hearts (Johansen and Burggren, 1984; Burggren et al., 2014), may have been compromised at higher rates because the atrial preparations exhibited a negative force–frequency effect over these frequencies (Fig. 3B).

Noradrenaline increased heart rate to 47.4 ± 1.6 beats min^{-1} , which did not differ statistically from pacing at 0.83 Hz (Fig. 1). Noradrenaline further decreased preload pressure, but maintained stroke volume, permitting an increased cardiac output (Fig. 1). In the Starling trials, noradrenaline vastly increased cardiac output and stroke volume across all preload pressures (Fig. 2). Atrial and ventricular preparations increased force in response noradrenaline, and also the rates of contraction and relaxation were faster across all frequencies (Fig. 3).

The positive inotropic effect of noradrenaline on ventricular myocardium suggests that adrenergic stimulation likely decreased ESV in the working heart. However, the end-systolic reserve may be somewhat limited as ejection fractions are considered to be high in ectotherms (Jensen et al., 2016). Noradrenaline may also have aided cardiac filling by promoting the suctional filling of the ventricle, as indicated in an early study on excised turtle ventricles (Kraner, 1959), the large fall in preload pressure, and the positive lusitropic effect we observed in the *in vitro* studies. The force developed by atrial preparations at 48 beats min^{-1} in the presence of noradrenaline exceeded that at 36 beats min^{-1} in untreated *in vitro* preparations, thus any limitation on atrial filling of the ventricle was also negated by adrenergic stimulation (Fig. 3).

We adopted an *in situ* perfused cardiac preparation to specifically address the inherent capacity of the heart to circumvent autoregulation of cardiac output during adrenergic stimulation. In doing so, we neglected vascular regulation. As espoused by Guyton (1955), cardiac output must equal venous return, thus whilst sympathetic stimulation increases cardiac output, it must also promote venous return. Pertinent to our study, whilst noradrenaline increased cardiac output, it decreased preload pressure. This contrasts with the *in vivo* situation, wherein alpha-adrenoceptor-mediated vasoconstriction increases central venous pressure and promotes filling of the heart in snakes (Skals et al., 2006) and other

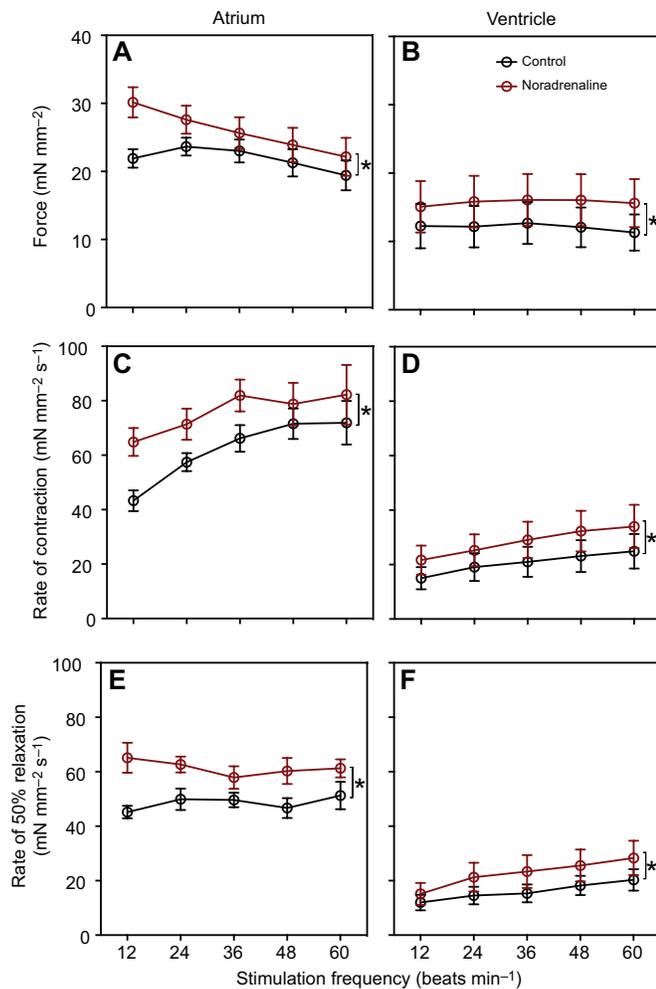


Fig. 3. Adrenergic stimulation of contractile performance in *in vitro* preparations from the anaconda heart. The effects of noradrenaline ($10 \mu\text{mol l}^{-1}$) on (A,B) the force of contraction, (C,D) rate of contraction and (E,F) rate of 50% relaxation during a force–frequency trial in atrial and ventricular preparations from the anaconda heart. Asterisks indicate a significant overall effect of noradrenaline across all frequencies (repeated-measures two-way ANOVA). Values are means \pm s.e.m. ($N=5$).

vertebrates (Sandblom et al., 2005). Whilst stroke volume was maintained by noradrenaline under steady-state conditions, at a given preload pressure it was increased. Thus, *in vivo*, the combination of increased contractility and increased venous return increases the scope for cardiac output further than either mechanism alone (Guyton, 1955).

It is noteworthy that in a similar study on perfused crocodile hearts, adrenaline produced an increase in heart rate that was offset by decreased stroke volume (Axelsson and Franklin, 1995). Adrenaline also had little effect on the sensitivity of cardiac output to preload pressure, suggesting that the crocodile myocardium has a blunted inotropic response to adrenaline. Nonetheless, we observed that cardiac output was increased by pacing if preload pressure was compensated, suggesting that autoregulation can also be circumvented (to a lesser extent) irrespective of the contractile state of the heart. Thus, to attain variable cardiac output, different species may be more dependent on regulation of venous return than on contractility.

Although stroke volume appears relatively static *in vivo*, the altered filling conditions at different heart rates mean that this can

only be achieved with extrinsic regulation. We demonstrate that the positive inotropic effect of noradrenaline is needed to complement its positive chronotropic effect to increase cardiac output. Adrenergic stimulation thus plays a fundamental role in increasing cardiac contractility, even in circumstances such as exercise, when frequency modulation of cardiac output appears to predominate.

Acknowledgements

We wish to thank Hans Gesser, who generously allowed unrestricted access to the equipment used for the *in vitro* experiments.

Competing interests

The authors declare no competing or financial interests.

Author contributions

W.J. conceived and designed the study, conducted the experiments, analysed the data and wrote the manuscript. M.A. and T.W. contributed to the experimental design, provided materials and gave input on the manuscript.

Funding

This study was funded by the Danish Research Council (Natur og Univers, Det Frie Forskningsråd).

References

- Altimiras, J. and Axelsson, M. (2004). Intrinsic autoregulation of cardiac output in rainbow trout (*Oncorhynchus mykiss*) at different heart rates. *J. Exp. Biol.* **207**, 195–201.
- Axelsson, M. and Franklin, C. (1995). The role of the pericardium and the effects of adrenaline and changes in oxygen tension on the performance of an *in situ* perfused crocodile heart. *J. Exp. Biol.* **198**, 2509–2518.
- Bevegård, B. S. and Shepherd, J. T. (1967). Regulation of the circulation during exercise in man. *Physiol. Rev.* **47**, 178–213.
- Burggren, W. W., Christoffels, V. M., Crossley, D. A., Enok, S., Farrell, A. P., Hedrick, M. S., Hicks, J. W., Jensen, B., Moorman, A. F. M., Mueller, C. A. et al. (2014). Comparative cardiovascular physiology: future trends, opportunities and challenges. *Acta Physiol.* **210**, 257–276.
- Enok, S., Leite, G. S. P. C., Leite, C. A. C., Gesser, H., Hedrick, M. S. and Wang, T. (2016). Improved cardiac filling facilitates the postprandial elevation of stroke volume in *Python regius*. *J. Exp. Biol.* **219**, 3009–3018.
- Farrell, A. P. (1991). From hagfish to tuna: a perspective on cardiac function in fish. *Physiol. Zool.* **64**, 1137–1164.
- Farrell, A. P., Small, S. and Graham, M. S. (1989). Effect of heart rate and hypoxia on the performance of a perfused trout heart. *Can. J. Zool.* **67**, 274–280.
- Farrell, A., Franklin, C., Arthur, P., Thorarensen, H. and Cousins, K. (1994). Mechanical performance of an *in situ* perfused heart from the turtle *Chrysemys scripta* during normoxia and anoxia at 5°C and 15°C. *J. Exp. Biol.* **191**, 207–229.
- Franklin, C. and Axelsson, M. (1994). The intrinsic properties of an *in situ* perfused crocodile heart. *J. Exp. Biol.* **186**, 269–288.
- Galli, G. L. J., Gesser, H., Taylor, E. W., Shiels, H. A. and Wang, T. (2006). The role of the sarcoplasmic reticulum in the generation of high heart rates and blood pressures in reptiles. *J. Exp. Biol.* **209**, 1956–1963.
- Guyton, A. C. (1955). Determination of cardiac output by equating venous return curves with cardiac response curves. *Physiol. Rev.* **35**, 123–129.
- Jensen, B., Moorman, A. F. M. and Wang, T. (2014). Structure and function of the hearts of lizards and snakes. *Biol. Rev. Camb. Philos. Soc.* **89**, 302–336.
- Jensen, B., Agger, P., de Boer, B. A., Oostra, R.-J., Pedersen, M., van der Wal, A. C., Nils Planken, R. and Moorman, A. F. M. (2016). The hypertrabeculated (noncompacted) left ventricle is different from the ventricle of embryos and ectothermic vertebrates. *Biochim. Biophys. Acta* **1863**, 1696–1706.
- Johansen, K. and Burggren, W. W. (1984). Venous return and cardiac filling in varanid lizards. *J. Exp. Biol.* **113**, 389–399.
- Joyce, W., Gesser, H. and Wang, T. (2014). Purinergic receptors exert negative inotropic effects on the heart in all major groups of reptiles. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **171**, 16–22.
- Joyce, W., Axelsson, M., Altimiras, J. and Wang, T. (2016). *In situ* cardiac perfusion reveals interspecific variation of intraventricular flow separation in reptiles. *J. Exp. Biol.* **219**, 2220–2227.
- Kramer, J. C. (1959). Effects of increased residual volume, increased output resistance and autonomic drugs on ventricular suction in the turtle. *Circ. Res.* **7**, 101–106.
- Markwalder, J. and Starling, E. H. (1914). On the constancy of the systolic output under varying conditions. *J. Physiol.* **48**, 348–356.
- Munch, G. D. W., Svendsen, J. H., Damsgaard, R., Secher, N. H., González-Alonso, J. and Mortensen, S. P. (2014). Maximal heart rate does not limit

- cardiovascular capacity in healthy humans: insight from right atrial pacing during maximal exercise. *J. Physiol.* **592**, 377–390.
- Noble, M. I. M., Trenchard, D. and Guz, A.** (1966). Effect of changing heart rate on cardiovascular function in the conscious dog. *Circ. Res.* **19**, 206–213.
- Sandblom, E., Farrell, A. P., Altimiras, J., Axelsson, M. and Claireaux, G.** (2005). Cardiac preload and venous return in swimming sea bass (*Dicentrarchus labrax* L.). *J. Exp. Biol.* **208**, 1927–1935.
- Secor, S. M., Hicks, J. W. and Bennett, A. F.** (2000). Ventilatory and cardiovascular responses of a python (*Python molurus*) to exercise and digestion. *J. Exp. Biol.* **203**, 2447–2454.
- Shiels, H. A., Vornanen, M. and Farrell, A. P.** (2002). The force–frequency relationship in fish hearts – a review. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **132**, 811–826.
- Skals, M., Skovgaard, N., Abe, A. S. and Wang, T.** (2005). Venous tone and cardiac function in the South American rattlesnake *Crotalus durissus*: mean circulatory filling pressure during adrenergic stimulation in anaesthetised and fully recovered animals. *J. Exp. Biol.* **208**, 3747–3759.
- Skals, M., Skovgaard, N., Taylor, E. W., Leite, C. A. C., Abe, A. S. and Wang, T.** (2006). Cardiovascular changes under normoxic and hypoxic conditions in the air-breathing teleost *Synbranchus marmoratus*: importance of the venous system. *J. Exp. Biol.* **209**, 4167–4173.
- Wang, T., Taylor, E. W., Andrade, D. and Abe, A. S.** (2001). Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*. *J. Exp. Biol.* **204**, 3553–3560.
- Wang, T., Altimiras, J. and Axelsson, M.** (2002). Intracardiac flow separation in an in situ perfused heart from Burmese python *Python molurus*. *J. Exp. Biol.* **205**, 2715–2723.