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SHORT COMMUNICATION

Net cardiac shunts in anuran amphibians: physiology or physics?

Stanley S. Hillman[1,*], Michael S. Hedrick[2] and Zachary F. Kohl[2]

ABSTRACT

Amphibians have a single ventricle and common conus arteriosus that produces an equal pressure to the parallel pulmocutaneous and systemic vascular circuits. The distribution of blood flows between the pulmocutaneous ($\dot{Q}_{pul}$) and systemic ($\dot{Q}_{sys}$) circuits (net cardiac shunt) varies with a number of environmental conditions and behaviours; although autonomic regulation of pulmonary vascular resistance conductance has been emphasized, little attention has been paid to the possible contribution of the passive physical characteristics of the two circuits to pressure changes associated with variation in cardiac output. In this study, we re-analysed three recent studies that recorded net cardiac shunts in the cane toad (*Rhinella marina*) under a variety of conditions and treatments. In all three studies, $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ were linearly related to cardiac output ($\dot{Q}_{tot}$), but the slope was threefold higher for $\dot{Q}_{pul}$ compared with $\dot{Q}_{sys}$ as predicted by relative conductance increases associated with increases in pressure from perfused preparations where autonomic regulation and humoral control were eliminated. Our analysis indicates that the net cardiac shunt in the cane toad is predicted primarily by the physical, rather than physiological, characteristics of the parallel pulmonary and systemic vascular circuits.

KEY WORDS: *Rhinella marina*, Cardiovascular, Blood flow

INTRODUCTION

The amphibian cardiovascular system is characterized by having a single ventricle, generating equal pressure output that drives blood through the common conus arteriosus and into parallel systemic and pulmocutaneous circuits (Wang et al., 1999). Oxygenated blood from the lungs returns to the left atrium and passes through to the single ventricle, whereas systemic venous blood returns to the right atrium. In the single, undivided ventricle, systemic venous blood can re-enter the systemic circulation (right-to-left shunt), while pulmonary venous blood can re-enter the pulmonary circulation (left-to-right shunt) (Wang et al., 1999). Despite the lack of anatomical separation between these two sources of blood entering the single ventricle, there is ample evidence for effective flow separation within the amphibian ventricle that can minimize admixture of oxygen (Wang et al., 1999), although the amount of admixture varies with different metabolic states (Hedrick et al., 1999).

The parallel vascular arrangement of amphibian circulatory anatomy does not necessarily predict equal flow rates to each circuit but, rather, allows for a wide range of differential outputs. The distribution of flows should be determined by their respective conductances, as the pressure driving force is the same for each circuit. The variation in outflow between the pulmonary and systemic circuits is defined as the net cardiac shunt and is quantified by measuring the difference in volume flow rate between the pulmonary ($\dot{Q}_{pul}$) and systemic ($\dot{Q}_{sys}$) outflow vessels from the heart (Hedrick et al., 1999; Gamperl et al., 1999; Andersen et al., 2003). A net cardiac shunt of various magnitudes in anuran amphibians has been quantified with measurements of $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ and is dependent upon metabolic state or other conditions (Hedrick et al., 1999; Gamperl et al., 1999; Wang et al., 1999; Andersen et al., 2003).

There are two possible, but not mutually exclusive, explanations for variations in the net cardiac shunt between the systemic and pulmonary circuits with variation in cardiac output. The first involves direct autonomic regulation of the tonus of the respective vasculatures. In particular, regulation of pulmonary conductance by vagal (parasympathetic) innervation of the pulmonary artery has received considerable attention (West and Burggren, 1984; Gamperl et al., 1999). The second entails the passive properties of the vascular beds to perfusion pressures resulting from variation in cardiac output (West and Smits, 1994; Kohl et al., 2013). In this case, the physical characteristics of the systemic and respiratory vascular beds determine the conductance of, and flow through, each circuit. This mechanism is independent of direct autonomic regulation of conductance. Autonomic regulation and passive properties of the vascular circuits can occur in concert with each other, but most studies have considered only direct autonomic regulation of vascular tone as the basis for net cardiac shunts (but see West and Smits, 1994). Ultimately, both are the result of differences in autonomic regulation, one directly and the other mediated indirectly via autonomic control of cardiac output. We suggest that differences in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ are not sufficient to define direct autonomic regulation of the net cardiac shunt in amphibians unless it can be demonstrated that the differences are distinct from the physical characteristics of the indirect passive pressure-mediated effects on conductance in the two circuits. Our recent study examined the *in situ* physical characteristics of the pulmonary and systemic circuits in a variety of amphibians that were anesthetized and cranially pithed and perfused with only Ringers solution (Kohl et al., 2013). This protocol eliminated the possibility of direct autonomic regulation of tonus on the vasculature either through direct innervation or via humoral agents. Our analysis compares *in vivo* data for net cardiac shunts in cane toads from the literature with the results of our previous study (Kohl et al., 2013).

Physical characteristics of systemic and pulmonary circuits

The heart ejects a volume of blood per unit time, and the pressure initially developed for each circuit is dependent upon the volume ejected per unit time and the total conductance of the parallel circuits. Because input pressure to the vasculature increases with cardiac output from a single ventricle, the input pressure each circuit initially experiences in the conus arteriosus is equivalent. Previous studies with anurans have shown that the pressures experienced by the pulmonary and systemic circuits are identical (Jones and

[1]Department of Biology, Portland State University, Portland, OR 97207-0751, USA. [2]Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA.

*Author for correspondence (hillmans@pdx.edu)

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Shelton, 1972; Langille and Jones, 1977). The pressure in each circuit also influences the diameter, and hence the conductance, of each vascular circuit. There is no *a priori* reason to expect that pulmonary and systemic conductances are equal; therefore, it is unlikely that $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ should be equal (i.e. no net cardiac shunt) under most conditions. In fact, equal $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ is likely an unusual situation for amphibians, considering the parallel anatomical design and differences in vascular conductances between the systemic and pulmonary circuits. This is supported by a variety of studies (West and Smits, 1994; Hedrick et al., 1999; Gamperl et al., 1999; Andersen et al., 2003) that have reported equal flows in systemic and pulmonary circuits in only three of 23 independent treatments. To understand whether direct physiological regulation modifies vascular conductance and blood flow rates (direct autonomic regulation), a comparison is necessary with an appropriate control state where no negative feedback regulation is being exerted upon the vasculature. The presence of direct physiological control can only be demonstrated if the measured flows are different from those predicted by the physical properties of each vascular circuit to variations of input pressure (i.e. indirect passive physical properties), and not merely differences between $\dot{Q}_{sys}$ and $\dot{Q}_{pul}$.

RESULTS AND DISCUSSION

To evaluate whether active physiological control explains the variation in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$, it is necessary to evaluate both the physical, passive characteristics of the vasculature to variation in flow and pressure, and the changes in flows *in vivo* during variation in cardiac output. In our recent study (Kohl et al., 2013), we measured the passive physical characteristics of the vascular circuits for cane toads, *Rhinella marina* (Linnaeus 1758) (formerly *Bufo marinus*). The effect of variation in flow rate on the input pressure and conductances was quantified *in situ* for the pulmonary and systemic vasculature in anesthetized, cranially pithed toads that eliminated autonomic regulation of the vasculature. In that study, the change in flow rate of the pulmonary and systemic vascular circuits was linearly related to the input pressure (Fig. 1A). The range of flow rates that we used encompassed the range of known flow rates for toads *in vivo* (Hedrick et al., 1999). The slope of this relationship was 2.7 times greater in the pulmonary circuit compared with the systemic circuit, indicating a greater inherent pressure effect on conductance of the pulmonary circuit. The nearly threefold increase in conductance of the pulmonary circuit reflects the passive mechanical effects of pressure on vascular distension, increasing conductance of each vascular network. This represents the passive control state for comparison of *in vivo* results that attempt to quantify direct autonomic control of the net cardiac shunt.

There are three studies that report *in vivo* data describing net cardiac shunt flow in *R. marina*, and we have re-analysed the data from these studies in light of the passive physical characteristics of the pulmonary and systemic vascular circuits described in our 2013 study (Kohl et al., 2013). Hedrick et al. (Hedrick et al., 1999) quantified *in vivo* variation in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ at rest and during exercise at temperatures ranging from 10 to 30°C. In their study, net cardiac shunt ranged from 24 ml min^{-1} kg^{-1} for resting animals at 10°C to 221 ml min^{-1} kg^{-1} for animals exercising at 30°C. In the six reported treatments from their study, no treatment produced a condition in which $\dot{Q}_{pul}$ was equal to $\dot{Q}_{sys}$ (i.e. no net cardiac shunt). Indeed, the net cardiac shunt was significantly correlated with total cardiac output ($\dot{Q}_{tot}$), suggesting there were larger increases in $\dot{Q}_{pul}$ relative to $\dot{Q}_{sys}$ over a wide range of activity states (Hedrick et al., 1999). The results also suggested that there are proportionally larger increases in conductance of the pulmonary circuit relative to the systemic circuit. When blood flows are expressed relative to $\dot{Q}_{tot}$, there is an approximately 3:1 increase in $\dot{Q}_{pul}$ relative to $\dot{Q}_{sys}$ (Fig. 1B, Table 1) with changes in $\dot{Q}_{tot}$, which is statistically identical to that described with *in situ* variations in pressure (Kohl et al., 2013) and consistent with proportionally larger increases in pulmonary conductance relative to systemic conductance with increased pressure.

Andersen et al. (Andersen et al., 2003) described the effects of decreased arterial P_{O_2} and isovolumetric anemia on $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ in resting *R. marina*. These treatments also changed $\dot{Q}_{tot}$, and when the combined data are plotted with variation in $\dot{Q}_{tot}$, $\dot{Q}_{pul}$ increases ~3 times faster than $\dot{Q}_{sys}$ (Fig. 1C). In only one of 10 treatment groups was $\dot{Q}_{pul}$ equal to $\dot{Q}_{sys}$. These results are also consistent with the net cardiac shunts being predicted by the passive mechanical effects of cardiac output on pressure and conductance rather than variation in flows determined by direct autonomic regulation of the net cardiac shunt.

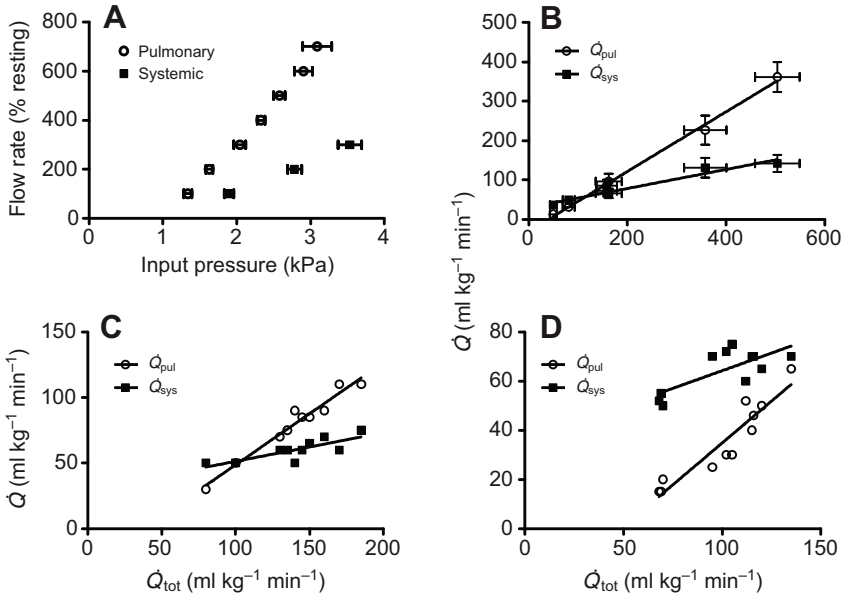

Fig. 1. Effects of pressure and cardiac output on pulmonary and systemic blood flow rates in *Rhinella marina*. (A) The relationship between percentage change in flow rate and input pressure resulting from changes in the perfusion rate for pulmonary blood flow rate ($\dot{Q}_{pul}$) and systemic blood flow rate ($\dot{Q}_{sys}$) (from Kohl et al., 2013). (B) Effects of *in vivo* variation in cardiac output ($\dot{Q}_{tot}$) on blood flow in the pulmonary ($\dot{Q}_{pul}$) and systemic ($\dot{Q}_{sys}$) vasculature. Data taken from Hedrick et al. (Hedrick et al., 1999). (C) Effects of *in vivo* variation in $\dot{Q}_{total}$ on $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$. Data taken from Andersen et al. (Andersen et al., 2003). (E) Effects of *in vivo* variation in $\dot{Q}_{total}$ on $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$. Data taken from Gamperl et al. (Gamperl et al., 1999). Slopes of each relationship are found in Table 1.

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Table 1. Pulmonary and systemic regression comparisons

Variable		Andersen et al., 2003	Gamperl et al., 1999	Hedrick et al., 1999	Mean	Kohl et al., 2013 (% change)	ANOVA result
$\dot{Q}_{pul}$	Slope	0.78±0.057	0.68±0.085	0.76±0.024	0.74	333	$F_{2,21}$=0.4; P=0.67
	b	−29.4	−33.2	−30.1	−30.9	−357	–
	r^2	0.96	0.88	0.99	–	0.99	–
$\dot{Q}_{sys}$	Slope	0.22±0.057	0.29±0.085	0.24±0.057	0.25	122	$F_{2,21}$=0.17; P=0.85
	b	29.4	35.6	30.1	31.7	−134	–
	r^2	0.65	0.56	0.96	–	0.99	–
$\dot{Q}_{pul}$: $\dot{Q}_{sys}$		3.5	2.4	3.1	3.0	2.7	–

Values are means (±s.e.m.), where appropriate, and the slope of each individual relationship was significantly non-zero (P<0.05).
The data of Kohl et al. (Kohl et al., 2013) were measured as percentage change in $\dot{Q}_{pul}$ or $\dot{Q}_{sys}$ relative to input pressure of the perfusion system.

Finally, Gamperl et al. (Gamperl et al., 1999) report $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ for conscious, resting toads exposed to hypoxia and hypercapnia at two temperatures (15 and 25°C) and after eliminating cholinergic tone on the vasculature with atropine. Pulmonary and systemic flow rates were equivalent in only two of seven treatments. Again, when these data are summarized as a function of $\dot{Q}_{tot}$, there is an approximate 3:1 increase in $\dot{Q}_{pul}$ relative to $\dot{Q}_{sys}$ (Fig. 1D, Table 1), consistent with indirect passive, rather than direct active, autonomic conductance changes determining $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$. Their study also showed a clear change in net cardiac shunt with removal of parasympathetic tone by atropine injection, which illustrates the difficulty of separating passive physical effects from autonomic regulation of net cardiac shunt (see below).

In all three studies, the changes in blood flow distribution were interpreted as being the result of direct autonomic regulation altering the outflow conductances. The slopes of the three studies relating $\dot{Q}_{pul}$ or $\dot{Q}_{sys}$ to cardiac output were identical (Table 1; $\dot{Q}_{pul}$: $F_{2,21}$=0.4; P=0.67 and $\dot{Q}_{sys}$: $F_{2,21}$=0.2; P=0.85). The control state, based on the passive physical response of the vasculature to pressure alone (Kohl et al., 2013), predicts that $\dot{Q}_{pul}$ should increase 2.7 times faster than $\dot{Q}_{sys}$, with increases in pressure associated with increased cardiac output. The actual ratios of increases in pulmonary blood flows to systemic flows with increased cardiac output are statistically indistinguishable from this physical prediction in the three studies used for comparison (Table 1). This suggests that the net cardiac shunt in toads primarily reflects the indirect passive physical properties of the respective vasculatures based on the dynamic conductance of the pulmonary and systemic circuits with pressure. Previous work with conscious toads also suggested that variations in $\dot{Q}_{pul}$ relative to $\dot{Q}_{sys}$ with increased $\dot{Q}_{tot}$ might be partially mediated by passive components of the vascular circuits (West and Smits, 1994). This conclusion was based on earlier work that noted an increase in pulmonary conductance when total flow increased in anesthetized toads, suggesting a distension of pulmonary capillaries in response to increased pressure (West and Smits, 1994).

Our conclusions do not suggest that active regulation of the net cardiac shunt cannot occur. Physiological control over blood flow, particularly in the pulmonary circuit, has been clearly demonstrated in amphibians (Emilio and Shelton, 1972; West and Burggren, 1984; West and Smits, 1994). In the present analysis, it is clear that examining blood flow over a large range is necessary to observe the 3:1 ratio for $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ with respect to $\dot{Q}_{tot}$, and variation around the mean slopes is apparent. In statistical terms, the variation in $\dot{Q}_{pul}$ and $\dot{Q}_{sys}$ that is explained by $\dot{Q}_{tot}$ (i.e. r^2) is not equal (Table 1) and is likely due to differences in treatments, measurement conditions and inherent error in measurements. This is in contrast to the strong correlation and low variation (r^2=0.99) in perfused toads under highly controlled conditions where autonomic tone was eliminated (Fig. 1A) (Kohl et al., 2013). As pointed out above, the change in net cardiac shunt with removal of parasympathetic tone (Gamperl et al., 1999) is an example of autonomic regulation of blood flow distribution. However, the changes in flow are small relative to the total range of flows, and it is clear that examining a limited range of flows would not consistently yield a 3:1 ratio of $\dot{Q}_{pul}$ to $\dot{Q}_{sys}$. Thus, it is possible that the variation around the mean slopes represents the direct active autonomic component of the net cardiac shunt. However, the analysis presented here suggests that what has been interpreted as direct physiological regulation of blood flow distribution in amphibians appears, instead, to be primarily a physical system in which pulmonary and systemic flows are modulated by variations in cardiac output. A larger question is to what extent the physical characteristics of the pulmonary and systemic circuits are responsible for net cardiac shunts in other amphibians and reptiles that have a single ventricle. Unfortunately, there are few studies that have quantified the relative intrinsic conductances of the pulmonary and systemic vasculatures to output induced pressure changes necessary to distinguish between physical and physiological regulation of net cardiac shunts. Until more data become available, the precise mechanisms of net cardiac shunts in amphibians and reptiles remain unclear.

MATERIALS AND METHODS

Least square linear regression was used to determine slopes of the relationships of pulmonary and systemic blood flows with cardiac output. All the relationships were highly significant. Comparison of slopes was via ANOVA. All statistical comparisons were done with Prism v. 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors contributed equally to the development and writing of the manuscript.

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