Baroreflex function in anurans from different environments

Michael S. Hedrick*, Kadi A. McNew, Dane A. Crossley II

Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA

ARTICLE INFO

Article history:

Received 3 August 2014

Received in revised form 3 October 2014

Accepted 3 October 2014

Available online 16 October 2014

Keywords:

Blood pressure

Heart rate

Rhinella marina

Lithobates catesbeianus

Xenopus laevis

ABSTRACT

Anurans from terrestrial environments have an enhanced ability to maintain mean arterial blood pressure (P_m) through lymph mobilization in response to desiccation or hemorrhage compared with semiaquatic or aquatic species. Because short term blood pressure homeostasis is regulated by arterial baroreceptors, we compared baroreflex function in three species of anurans that span a range of environments, dehydration tolerance and an ability to maintain P_m with dehydration and hemorrhage. The cardiac limb of the baroreflex loop was studied using pharmacological manipulation of P_m with phenylephrine and sodium nitroprusside ($20\text{--}200\text{ }\mu\text{g kg}^{-1}$), and the resulting changes in heart rate (f_H) were quantitatively analyzed using a four-parameter sigmoidal logistic function. Resting P_m in the aquatic species, *Xenopus laevis*, was 3.6 ± 0.3 kPa and was significantly less ($P < 0.005$) than for the semiaquatic species, *Lithobates catesbeianus* (4.1 ± 0.2 kPa), or the terrestrial species, *Rhinella marina* (4.7 ± 0.2 kPa). The maximal baroreflex gain was not different among the three species and ranged from 12.1 to 14.3 beats min^{-1} kPa^{-1} and occurred at P_m ranging from 3.0 to 3.8 kPa, which were slightly below the resting P_m for each species. Mean arterial blood pressures at rest in the three species were near the saturation point of the baroreflex curve which provides the animals with a greater f_H response range to hypotensive, rather than hypertensive, changes in P_m . This is consistent with the hypothesis that arterial baroreceptors are key sensory components that allow anurans to maintain P_m possibly by mobilization of lymphatic return in response to hypotension.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Anuran amphibians are remarkable for their ability to withstand desiccation or hemorrhagic blood loss that would be lethal to other vertebrates. Within anurans, this ability correlates strongly with habitat; that is, more terrestrial species have a greater tolerance for desiccation or hemorrhage compared with semi-aquatic or aquatic species (Hillman, 1987; Hillman and Withers, 1988; Hillman et al., 2010a). The ability of terrestrial species to withstand greater water or plasma loss is due to their ability to maintain arterial blood pressure and blood volume homeostasis (Hillman et al., 2010a). It has become clear that the key to maintenance of arterial blood pressure and blood volume is mobilization of lymphatic return to the circulation (Hillman et al., 2004, 2010b, 2011).

The presence of a baroreflex in anuran amphibians is well established (Millard and Moalli, 1980; Smith et al., 1981; Hoffmann and Cordeiro de Souza, 1982; West and Van Vliet, 1983, 1992, 1994; Van Vliet and West, 1987a,b). Several studies have shown that anuran amphibians have arterial baroreceptors in a variety of locations within the aortic trunk (Neil et al., 1950; Bagshaw, 1985; West and Van Vliet,

1992; Bianchi-da-Silva et al., 2000) that converge on the nucleus tractus solitarius (NTS), but the primary receptors are located within the walls of the pulmocutaneous artery (Ishii and Ishii, 1978) and respond to increased arterial pressure with increased discharge activity (Van Vliet and West, 1987a,b). However, baroreceptor unloading with sodium nitroprusside (SNP) suggested that baroreceptors in the aortic region were primarily responsible for the reflex tachycardia in response to the reduction in peripheral resistance induced by SNP (Bianchi-da-Silva et al., 2000). Previous work with anurans indicated that the maximum open-loop gain of the baroreflex occurred at arterial pressures higher than the normal pulmocutaneous pressures, which was interpreted to suggest that the baroreflex was more effective at guarding against an elevation in pressure to protect the lungs from excessive fluid loss in pulmonary capillaries (West and Van Vliet, 1983; Smits et al., 1986). However, these studies relied on extensive surgical instrumentation that may have compromised the function in the preparation. In addition, anurans have very 'leaky' capillaries and, coupled with the highest interstitial compliance among vertebrates, any elevation in pressure is likely to produce large amounts of filtered plasma (i.e. lymph) (Hillman et al., 2010a). Indeed, Starling forces cannot account for fluid balance at the capillary level and trans-capillary hydrostatic forces favor efflux of plasma from the vascular to the interstitial space under all conditions including dehydration (Hillman et al., 1987). Thus, it seems unlikely that the baroreflex operates as a 'protective' mechanism against excessive pressures and pulmonary edema.

* Corresponding author at: Department of Biological Sciences, California State University, East Bay, Hayward, CA, USA. Tel.: +1 510 885 7443; fax: +1 510 885 4747.
E-mail address: michael.hedrick@csueastbay.edu (M.S. Hedrick).

Given the importance of the lymphatic system for maintaining arterial blood pressure, we hypothesize that the arterial baroreflex operates primarily to sense and respond to reductions in arterial blood pressure to provide a signal to the lymphatic effectors (lung ventilation and skeletal muscles) to mobilize lymph in order to restore blood pressure and blood volume. With this background, we have examined the *in vivo* baroreflex responses in three anuran species (*Xenopus laevis*, *Lithobates catesbeianus* and *Rhinella marina*) that represent a range of habitats from completely aquatic to terrestrial. Our hypothesis was that the more terrestrial species would have a greater baroreflex sensitivity compared with the aquatic species that is consistent with previous findings that the lymphatic effectors exhibit habitat-related differences in function and morphology (Hedrick et al., 2011, 2013; Hillman et al., 2011; Drewes et al., 2007, 2013; Withers et al., 2014).

2. Materials and methods

2.1. Animals

A total of 21 animals were used in this study: 10 cane toads (*R. marina*; formerly *Bufo marinus*; mean mass = 266 ± 20 g), 6 North American bullfrogs (*L. catesbeianus*; formerly *Rana catesbeiana*; mean mass = 235 ± 41 g), and 5 African clawed frogs (*X. laevis*; mean mass = 156 ± 7 g). Animals were obtained from commercial suppliers. Animals were housed at 22 °C and all experiments were carried out at room temperature (20–22 °C). All procedures were approved by the UNT IACUC (protocol #11010).

2.2. Surgical preparation

Each animal was anesthetized by immersion in buffered tricaine methanesulfonate (MS 222; 0.3%) until reflex responses were abolished. For drug injection, the anterior (ventral) abdominal vein was cannulated with a 25–30 cm length of polyethylene (PE) 90 tubing. The vein was exposed by a ca. 0.5 cm incision through the skin and underlying musculature; the vein was occlusively cannulated in the retrograde direction and the cannula filled with heparinized (50 IU/ml) amphibian saline (0.8% NaCl). Following cannulation, the muscle and skin layers were separately sutured closed with 4-0 silk. For measurement of arterial blood pressure, a similar length of PE 50 tubing was used to cannulate a sciatic artery. The sciatic artery was exposed by a 0.5 cm incision in the skin on the dorsal aspect of the thigh followed by separating the fascia between the biceps and semimembranosus muscles, where the artery is found lying adjacent to the sciatic nerve. The artery was tied distally and the cannula inserted in the anterograde direction, filled with heparinized saline and the muscles and skin layers were separately sutured closed with 4-0 silk suture. Animals were allowed 24 h to recover from the surgery before experiments began. This recovery time is sufficient for anurans to restore acid-base status (Andersen and Wang, 2002) or resting heart rate (Zena et al., 2013) after anesthesia.

2.3. Protocol

Measurements of arterial pressure began after the 24 h recovery period. For bullfrogs and cane toads, each animal was placed in a temperature-controlled chamber regulated by a circulating water bath (Fisher Scientific) that was set at 21 (± 1) °C. For African clawed frogs, each animal was placed in a plastic container (1.5 l) with dechlorinated water and was visually isolated. Water temperature was measured at the start of an experiment and did not vary throughout the experiment. The range of water temperatures for all animals was 20–22 °C.

For each animal, the arterial cannula was connected to a pressure transducer that was calibrated with a static water column prior to each experiment. Blood pressure was measured for at least 1 h to provide a stable control value for resting blood pressure before drug

injections began. Heart rate was recorded on a separate channel by triggering the systolic peak intervals that occurred with each heartbeat. Data were acquired at 100 Hz in LabChart software (v. 7) running on Windows PC platform.

Following the control recording, serial increasing doses of sodium nitroprusside (SNP, Sigma; 20, 50, 100, 200 $\mu\text{g kg}^{-1}$) and phenylephrine hydrochloride (Phe, Sigma; 20, 50, 100, 200 $\mu\text{g kg}^{-1}$) in saline were injected via the ventral abdominal vein cannula while continuously recording arterial blood pressure and f_H . The injected drug (0.02 ml to 0.3 ml bolus) was flushed with approximately 0.2 ml saline. The next dose was not injected until the recorded variables had returned to pre-injection values, which normally occurred within 10 min except with largest doses of drug.

2.4. Data analysis

Measurements of control (pre-drug) and drug (Phe and SNP) injection values for heart rate (f_H) and mean arterial blood pressure (P_m) were extracted from the raw data using LabChart Pro software. To assess baroreflex function, we used a 'static' method which entailed measuring the maximum and minimum P_m and the corresponding maximum reflex changes in f_H after each drug injection. P_m was calculated as:

$$P_m = \frac{2}{3}P_D + \frac{1}{3}P_S$$

where P_D is diastolic pressure and P_S is the systolic pressure. The P_m and f_H values obtained across all Phe and SNP injections were plotted against each other for each individual animal. The corresponding P_m and f_H for each animal were analyzed using a four-variable sigmoidal logistic function (Kent et al., 1972; Reid, 1996):

$$f_H = \frac{(\max - \min)}{(1 + (P_m/C)^B)} + \min,$$

where max and min are the maximum and minimum f_H (beats min^{-1}), respectively, attained by the baroreflex, B is the maximum slope of the linear portion of the curve (beats $\text{min}^{-1} \text{ kPa}^{-1}$), and C is the P_m (kPa) when f_H is at the midpoint of its range. The best fit was determined using the quasi-Newtonian iterative method in the nonlinear estimation module of Statistica 10 (StatSoft, Tulsa, OK). The absolute value of the gain (G_{50}) of the baroreflex when $P_m = C$ was calculated for each animal as

$$G_{50} = \left| \left(\frac{-B(\max - \min)}{4C} \right) \right|.$$

For comparison between treatments, the gain was normalized as a percentage change in f_H per unit change in P_m (G ; %/kPa), and adapted to the four-variable sigmoidal model (Altimiras et al. 1998):

$$G = \frac{100B}{\min}.$$

Differences in baroreflex variables were tested with a one-way ANOVA and a Tukey HSD post-hoc test. $P < 0.05$ was taken as the minimum level of statistical significance.

3. Results

Pharmacologically-induced changes in blood pressure with Phe and SNP produced clear baroreflex responses (Fig. 1). Phenylephrine, an alpha-adrenergic antagonist, increased P_m through vasoconstriction of peripheral vascular vessels; the resultant increase in pressure is sensed by baroreceptors resulting in a reflex reduction in f_H (bradycardia). Sodium nitroprusside has the opposite response producing a general

Fig. 1. Original trace of arterial blood pressure (P_a ; kPa) from a single *L. catesbeianus* after injection of $50 \mu\text{g kg}^{-1}$ sodium nitroprusside (SNP) and $50 \mu\text{g kg}^{-1}$ phenylephrine (Phe). The arrows mark the time of injection. Note the increase of heart rate (f_H ; min^{-1}) with injection of SNP and reduction in f_H with injection of Phe.

vasodilation of peripheral vessels through the production of nitric oxide and a reflex tachycardia (Fig. 1).

Each animal responded to alterations in pressure with Phe or SNP with a reflex bradycardia or tachycardia, respectively. A baroreflex curve was calculated using the four parameter logistic model for each animal based on the change of pressure produced by injection of Phe and SNP and the corresponding f_H for each dose of drug (Fig. 2). Although there was considerable variation in the position of the curve, all animals exhibited a typical sigmoidal baroreflex curve (Fig. 2).

Average values for the baroreflex parameters, and control values for P_m and f_H , calculated from the sigmoidal logistic equation are shown in Table 1. Control P_m (3.5 ± 0.3 kPa) for *X. laevis* was significantly lower than P_m for *R. marina*, but not significantly different from *L. catesbeianus* (Table 1). Maximal gain of the baroreflex ranged from 12.1 ± 2.0 beats $\text{min}^{-1} \text{kPa}^{-1}$ in *L. catesbeianus* to 14.3 ± 2.7 beats $\text{min}^{-1} \text{kPa}^{-1}$ in *X. laevis*, but there was no significant difference among the three species. The maximal gain occurred at a P_m of 3.0 ± 0.2 kPa for *X. laevis* which was significantly less than the P_m for maximal gain in *R. marina* (3.8 ± 0.2 kPa) (Table 1). The values for P_m at maximal gain were less than the control values for P_m for each species which was also significantly less in *X. laevis* compared with *R. marina*.

A single average baroreflex curve was generated for each species using the individual curves and is shown in Fig. 3. The resting (control) values for mean blood pressure and heart rate are superimposed on the baroreflex curves. Although the resting P_m for *X. laevis* was significantly lower than for *R. marina*, all three species are near the saturation point on each curve. Although increases in blood pressure from resting values would produce reflex reductions in heart rate, there is a much greater scope for heart rate change (tachycardia) in response to reductions in mean pressure. In other words, because resting blood pressure is near the maximum slope of the baroreflex curve (Table 1), a given change in blood pressure in the hypotensive direction would produce a much larger change in heart rate than an equal change in blood pressure in the hypertensive direction (Fig. 3).

Fig. 2. Individual baroreflex curves for (A) *L. catesbeianus*, (B) *R. marina* and (C) *X. laevis* in response to dose-dependent changes in mean arterial pressure (P_m) and heart rate (f_H) (see text for details). Different symbols represent individual animals.

4. Discussion

Our initial hypothesis was that baroreflex sensitivity would be correlated with terrestriality in anurans; however, this hypothesis was not supported because baroreflex gain was equivalent in all three species (Table 1). We did find that the aquatic frog, *X. laevis*, had a lower resting blood pressure than *R. marina*, but all three species had similar resting

Table 1

Baroreflex variables for the three species used in this study (see Methods for definitions). Significant differences indicated by different superscript letters (a or b). See text for details. Values are mean $\pm$ s.e.m.

	<i>X. laevis</i>	<i>L. catesbeianus</i>	<i>R. marina</i>
max f_H , beats min^{-1}	47.1 $\pm$ 4.5	42.8 $\pm$ 3.8	46.2 $\pm$ 1.9
min f_H , beats min^{-1}	24.7 $\pm$ 1.6	18.4 $\pm$ 2.3	19.8 $\pm$ 1.5
B, beats $\text{min}^{-1} \text{kPa}^{-1}$	8.3 $\pm$ 1.2	7.4 $\pm$ 0.8	7.8 $\pm$ 0.9
C, kPa	3.0 $\pm$ 0.2 ^a	3.6 $\pm$ 0.2 ^b	3.8 $\pm$ 0.2 ^b
G ₅₀ , beats $\text{min}^{-1} \text{kPa}^{-1}$	14.3 $\pm$ 2.7	12.1 $\pm$ 2.0	13.2 $\pm$ 1.3
Gain, %/kPa	33.6 $\pm$ 4.2	42.5 $\pm$ 5.9	42.4 $\pm$ 6.6
Control P_m , kPa	3.5 $\pm$ 0.3 ^a	4.0 $\pm$ 0.2 ^b	4.4 $\pm$ 0.1 ^b
Control f_H , beats min^{-1}	27.8 $\pm$ 2.7	29.5 $\pm$ 3.6	29.2 $\pm$ 1.1

Fig. 3. Summary of the relationship between mean arterial blood pressure (P_m) and mean heart rate (f_H) for the three species used in this study. Filled symbols represent the resting (control) blood pressure and heart rate for each species (squares = *X. laevis*; diamonds = *L. catesbeianus*; circles = *R. marina*). Values are mean $\pm$ sem.

heart rates. Our major conclusion from this study is that all three species have resting arterial blood pressures near the saturation point of the baroreflex curve (Fig. 3) which would enable a greater heart rate change in response to a reduction in arterial blood pressure. This conclusion contrasts with previous work suggesting that the baroreflex in anurans is primarily responsible for protection of the lungs against hypertension-induced plasma flux across the pulmonary capillaries (West and Van Vliet, 1983; Smits et al., 1986). While the peripheral component of the baroreflex was not quantified in this study, the cardiac limb sensitivity appears to be species independent. In the context of lymph mobilization, and the role of specialized skeletal muscles and lung ventilation as effectors of lymph movement, the ability to respond to hypotension is likely more important for maintaining arterial blood pressure and blood volume in anurans.

The results of this study must be interpreted with caution since species used are distantly related phylogenetically (Pyron and Wiens, 2011) and differences may result from phylogenetic history rather than habitat. However, the only significant difference between the three species was a lower resting P_m and P_m at the mid-point of the heart rate range (C in Table 1) in *X. laevis* compared with *L. catesbeianus* and *R. marina*. It is interesting that phylogenetically distant groups of anurans exhibit very similar baroreflex responses perhaps suggesting homoplasy of baroreceptor function in anurans.

Although several studies have measured the baroreflex gain for anurans (Millard and Moalli, 1980; Smith et al., 1981; Van Vliet and West, 1989; West and Van Vliet, 1992), this is the first study to use multiple species of anurans in the same study and, to our knowledge, the first study to measure baroreflex function in the aquatic pipid, *X. laevis*. Our findings with a limited data set of three species indicate that terrestriality per se does not represent a significant driving force for the evolution of baroreflex function in anurans. However, examination of more species would be necessary to fully address this question. By contrast, lymph mobilization capabilities, lymph skeletal muscle function and pulmonary volume and compliance exhibit clear habitat-related differences (Hedrick et al., 2011; Hillman et al., 2011; Withers et al., 2014). It should be noted that our use of the Oxford method excludes the peripheral limb, or vascular component, of the baroreflex. Without additional experiments, definitive statements regarding differences in baroreflex function between these species cannot be conclusive. However, the arterial pressure set point of the reflex differed between species indicating that *X. laevis* at rest tolerates a low resting arterial pressure in comparison to the other two species.

We used the pharmacological 'Oxford' method, intravascular injection of SNP and Phe, to evoke the peripheral limb of the baroreflex in anurans. This method causes reductions in peripheral resistance (SNP) through vasodilation and increases in peripheral resistance (Phe) through vasoconstriction and is considered a 'closed-loop' method for characterizing the baroreflex. This contrasts with 'open-loop' method where changes in pressure are isolated to the pulmocutaneous artery and are manipulated independently of arterial pressure (eg. West and Van, 1983). Both approaches offer advantages and disadvantages. The advantage of the 'open-loop' method is that both the cardiac limb and the peripheral limb of the reflex are assessed, thus providing a more direct indicator of the total response of the reflex for compensating for changes in arterial pressure (Van Vliet and West, 1994). The biggest disadvantage is that animals are anesthetized and heavily instrumented to isolate the baroreceptive regions. This method also requires knowledge of the primary baroreceptive areas, which are not completely defined in many ectothermic vertebrates. The primary advantages of closed-loop studies are that animals are minimally instrumented and the pharmacologically-induced changes in arterial pressure are done with conscious animals, thus providing an integrated response of the cardiovascular system. The disadvantage is that only the cardiac limb of the baroreflex is assessed, thus potentially providing an underestimate of the baroreflex gain.

Changes in arterial pressure resulting from alterations in peripheral resistance are detected primarily by mechanosensitive nerve endings of the recurrent laryngeal nerve in the pulmocutaneous artery (Neil et al., 1950; Ishii and Ishii, 1978; Van Vliet and West, 1987a,b) that are transmitted to the brainstem resulting in reflex changes in heart rate. However, other vascular sites, including the carotid artery and aortic arch, that detect and respond to changes in arterial pressure have also been characterized in anurans (West and Van Vliet, 1992). Because changes in f_H and blood pressure reach lower and upper limits at either end of the range (i.e. threshold and saturation, respectively), there is a sigmoidal, rather than linear, relationship between f_H and P_m (Kent et al., 1972; Reid, 1996). Thus, statistical evaluation of this sigmoidal relationship represents a more accurate method for examining the baroreflex relationship than in previous work that used linear regression techniques. The use of a four-parameter logistic function to evaluate baroreflex function has only been applied to ectotherm data in recent years (see Altimiras et al., 1998; Crossley et al., 2003; Hagensen et al., 2010); thus it is difficult to make direct comparisons with earlier work that applied linear regression analysis (eg. Smith et al., 1981; Millard and Moalli, 1980). For example, our values for normalized gain for *R. marina* (42.4 %/kPa) are about three times higher than the gain reported previously using a closed-loop method for measuring baroreflex in this species (13 %/kPa; Smith et al., 1981) and for *L. catesbeianus* (13 %/kPa; Millard and Moalli, 1980). However, our value for normalized closed-loop gain in *R. marina* is nearly identical to the value of 39 %/kPa measured previously for this species when a third-order regression analysis was applied (Van Vliet and West, 1989).

The baroreflex in anurans is clearly linked to the lymphatic system. Previous work has shown that lymph heart rate is inversely related to peak systolic pressure (Crossley and Hillman, 1999) in *R. marina* and this relationship is abolished when the recurrent laryngeal nerve (RLN) is cut. In addition, stimulation of the RLN stops lymph heart activity (Crossley and Hillman, 1999) indicating that a fictive increase in arterial blood pressure, mimicked by RLN stimulation, provides an adaptive response to halt lymph mobilization. Preliminary evidence also suggests the baroreflex is also linked to pulmonary ventilation, one of the effectors of lymph mobilization, in *R. marina* (Hedrick et al., 2012). Unloading the baroreceptors with SNP increases lung ventilation which has been shown to mobilize lymph return in anurans (Hedrick et al., 2007; Hillman et al., 2010b). However, at this time it is unclear how the afferent limb of the baroreflex is integrated with brainstem structures to produce efferent effects on the lymphatic effectors of lung ventilation and lymph skeletal muscles. Future studies will be necessary to examine this linkage.

Acknowledgments

This work was supported by the National Science Foundation grants to MSH (IOS-0843082) and DAC (CAREER IOS-0845741). KAM was supported by the UNT-Howard Hughes Medical Institute Undergraduate Researchers (Grant number 52006955) Program.

References

- Altimiras, J., Franklin, C.E., Axelsson, M., 1998. Relationships between blood pressure and heart rate in the saltwater crocodile *Crocodylus porosus*. *J. Exp. Biol.* 201, 2235–2242.
- Andersen, J., Wang, T., 2002. Effects of anaesthesia on blood gases, acid-base status and ions in the toad *Bufo marinus*. *Comp. Biochem. Physiol.* 131A, 639–646.
- Bagshaw, R.J., 1985. Evolution of cardiovascular baroreceptor control. *Biol. Rev.* 60, 121–162.
- Bianchi-da-Silva, L.M., Menescal-de-Oliveira, L.M., Hoffmann, A., 2000. Baroreceptor control of heart rate in the awake toad: peripheral autonomic effectors and arterial baroreceptor areas. *J. Auton. Nerv. Syst.* 80, 31–39.
- Crossley II, D.A., Hillman, S.S., 1999. The role of pulmocutaneous baroreceptors in the control of lymphatic heart rate in the toad *Bufo marinus*. *Physiol. Biochem. Zool.* 72, 109–115.
- Crossley II, D.A., Hicks, J.W., Altimira, J., 2003. Ontogeny of baroreflex control in the American alligator, *Alligator mississippiensis*. *J. Exp. Biol.* 206, 2895–2902.
- Drewes, R.C., Hedrick, M.S., Hillman, S.S., Withers, P.C., 2007. Unique role of skeletal muscle contraction in vertical lymph movement in anurans. *J. Exp. Biol.* 210, 3931–3939.
- Drewes, R.C., Hillman, S.S., Hedrick, M.S., Withers, P.C., 2013. Evolutionary implications of the distribution and variation of the skeletal muscles of the anuran lymphatic system. *Zoomorphology* 132, 339–349.
- Hagensen, M.K., Abe, A.S., Wang, T., 2010. Baroreflex control of heart rate in the broad-nosed caiman *Caiman latirostris* is temperature dependent. *Comp. Biochem. Physiol.* 156, 458–462.
- Hedrick, M.S., Drewes, R.C., Hillman, S.S., Withers, P.C., 2007. Lung ventilation contributes to vertical lymph movement in anurans. *J. Exp. Biol.* 210, 3940–3945.
- Hedrick, M.S., Hillman, S.S., Drewes, R.C., Withers, P.C., 2011. Pulmonary compliance and lung volume varies with ecomorphology in anuran amphibians: implications for ventilatory-assisted lymph flux. *J. Exp. Biol.* 214, 3279–3285.
- Hedrick, M.S., Hillman, S.S., Drewes, R.C., Withers, P.C., 2012. Lung ventilation is an effector of the baroreflex in the cane toad (*Rhinella marina*). *FASEB J.* 26, 1071.10.
- Hedrick, M.S., Hillman, S.S., Drewes, R.C., Withers, P.C., 2013. Lymphatic regulation in non-mammalian vertebrates. *J. Appl. Physiol.* 115, 297–308.
- Hillman, S.S., 1987. Dehydrational effects on cardiovascular and metabolic capacity in two amphibians. *Physiol. Zool.* 60, 608–613.
- Hillman, S.S., Withers, P.C., 1988. The hemodynamic consequences of hemorrhage and hypernatremia in two amphibians. *J. Comp. Physiol. B.* 157, 807–812.
- Hillman, S.S., Zygmunt, A., Baustian, M., 1987. Transcapillary fluid forces during dehydration in two amphibians. *Physiol. Zool.* 60, 339–345.
- Hillman, S.S., Hedrick, M.S., Withers, P.C., Drewes, R.C., 2004. Lymph pools in the basement, sump pumps in the attic: the anuran dilemma for lymph movement. *Physiol. Biochem. Zool.* 77, 161–173.
- Hillman, S.S., DeGrauw, E.A., Hoagland, T.M., Hancock, T.V., Withers, P.C., 2010a. The role of vascular and interstitial compliance and vascular volume in the regulation of blood volume in two species of anuran. *Physiol. Biochem. Zool.* 83, 55–67.
- Hillman, S.S., Hedrick, M.S., Drewes, R.C., Withers, P.C., 2010b. Lymph flux rates from various lymph sacs in the cane toad *Rhinella marina*: an experimental evaluation of the roles of compliance, skeletal muscles and the lungs in the movement of lymph. *J. Exp. Biol.* 213, 3161–3166.
- Hillman, S.S., Drewes, R.C., Hedrick, M.S., Withers, P.C., 2011. Interspecific comparisons of lymph volume and lymphatic fluxes: do lymph reserves and lymph mobilization capacities vary in anurans from different environments? *Physiol. Biochem. Zool.* 84, 268–276.
- Hoffmann, A., Cordeiro de Souza, M.B., 1982. Cardiovascular reflexes in conscious toads. *J. Auton. Nerv. Syst.* 5, 345–355.
- Ishii, K., Ishii, K., 1978. A reflexogenic area for controlling the blood pressure of the toad (*Bufo vulgaris formosa*). *Jpn. J. Physiol.* 28, 423–431.
- Kent, B.B., Drane, J.W., Blumenstein, B.B., Manning, J.W., 1972. Mathematical model to assess changes in baroreceptor reflex. *Cardiology* 57, 295–310.
- Millard, R.W., Moalli, R., 1980. Baroreflex sensitivity in an amphibian, *Rana catesbeiana*, and a reptilian, *Pseudemys scripta elegans*. *J. Exp. Zool.* 213, 283–288.
- Neil, E.L., Strom, L., Zotterman, Y., 1950. Action potential studies of afferent fibers in the IXth and Xth cranial nerves of the frog. *Acta Physiol. Scand.* 20, 338–350.
- Pyron, R.A., Wiens, J.J., 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* 61, 543–583.
- Reid, I.A., 1996. Angiotensin II and baroreflex control of heart rate. *Physiology* 11, 270–274.
- Smith, D.G., Berger, P.J., Evans, B.K., 1981. Baroreceptor control of heart rate in the conscious toad *Bufo marinus*. *Am. J. Physiol.* 241, R307–R311.
- Smits, A.W., West, N.H., Burggren, W.W., 1986. Pulmonary fluid balance following baroreceptor denervation in the toad. *J. Appl. Physiol.* 61, 331–337.
- Van Vliet, B.N., West, N.H., 1987a. Responses to circulatory pressures, and conduction velocity, of pulmocutaneous baroreceptors in *Bufo marinus*. *J. Physiol.* 388, 41–53.
- Van Vliet, B.N., West, N.H., 1987b. Response characteristics of pulmocutaneous arterial baroreceptors in the toad, *Bufo marinus*. *J. Physiol.* 388, 55–70.
- Van Vliet, B.N., West, N.H., 1989. Cardiovascular responses to denervation of the pulmocutaneous baroreceptors in toads. *Am. J. Physiol.* 256, R946–R954.
- Van Vliet, B.N., West, N.H., 1994. Phylogenetic trends in the baroreceptor control of arterial pressure. *Physiol. Zool.* 67, 1284–1304.
- West, N.H., Van Vliet, B.N., 1983. Open-loop analysis of the pulmocutaneous baroreflex in the toad *Bufo marinus*. *Am. J. Physiol.* 245, R642–R650.
- West, N.H., Van Vliet, B.N., 1992. Sensory mechanisms regulating the cardiovascular and respiratory systems. In: Feder, M.E., Burggren, W.W. (Eds.), *Environmental Physiology of the Amphibians*. University of Chicago Press, Chicago, IL, pp. 151–182.
- West, N.H., Van Vliet, B.N., 1994. The role of arterial baroreceptors in the undivided circulation of anuran amphibians. *Physiol. Zool.* 67, 1305–1324.
- Withers, P.C., Hedrick, M.S., Drewes, R.C., Hillman, S.S., 2014. Pulmonary compliance and lung volume are related to terrestriality in anuran amphibians. *Physiol. Biochem. Zool.* 87, 374–383.
- Zena, L.A., Gargaglioni, L.H., Bicego, K.C., 2013. Role of brain nitric oxide in the cardiovascular control of bullfrogs. *Comp. Biochem. Physiol.* 165, 263–271.