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Baroreflex function in anurans from different environments

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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 (20–200 μg 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.

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 solitarii (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.

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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 μg kg^{-1}) and phenylephrine hydrochloride (Phe, Sigma; 20, 50, 100, 200 μg 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. Pm 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):

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

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 $\min^{-1}$ 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