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The interplay of cutaneous water loss, gas exchange and blood flow in the toad, *Bufo woodhousei:* adaptations in a terrestrially adapted amphibian

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Summary

Toads experiencing dehydrating conditions exhibit complex physiological and behavioral responses, some of which can potentially impact cutaneous gas exchange, an important component of total gas exchange. We measured the effect of dehydration on cutaneous gas exchange in the xeric-adapted toad *Bufo woodhousei*. First, two pharmacological agents were used to stimulate cutaneous blood flow – phentolamine (an α-blocker) and isoproterenol, a β-stimulant and powerful cardio-accelerator – to determine a relationship between cutaneous blood flow and water loss. Both drugs increased heart rate and blood pressure, and caused visually evident extensive vasodilation of the skin. Untreated toads in a dry air stream took an average of 10.1±0.7 h to dehydrate to 80% body mass, while animals treated with isoproterenol and phentolamine requires only 7.2±0.8 h and 7.4±0.9 h, respectively. Rehydration, which was more rapid than dehydration, was similarly accelerated in pharmacologically treated toads.

Cutaneous gas exchange ($\dot{M}_{O_2}$, $\dot{M}_{CO_2}$) and $C^{18}O$ diffusing capacity ($D_{Skin}C^{18}O$) were then examined in unanesthetized toads under different states of body hydration. Blood gases and hematocrit were measured separately but under identical conditions. In fully hydrated toads at 23–25°C, cutaneous gas exchange values were: $\dot{M}_{O_2}$ = 1.43±0.47 μmol g^{-1} h^{-1}, $\dot{M}_{CO_2}$ = 1.75±0.85 μmol g^{-1} h^{-1}, and the respiratory exchange ratio R = 1.36±0.56 (*N*=6, mean + 1S.D.). $D_{Skin}C^{18}O$ was 0.48±0.03 μmol g body $mass^{-1}$ h^{-1} kPa. Following an enforced 20–25% loss of body water, $D_{Skin}C^{18}O$ fell by nearly 50% to 0.28±0.09 μmol g^{-1} h^{-1} kPa. However, cutaneous $\dot{M}_{O_2}$, $\dot{M}_{CO_2}$ and R were unchanged at 1.48±0.15 μmol g^{-1} h^{-1}, 1.72±0.29 μmol g^{-1} h^{-1} and 1.13±0.08 μmol g^{-1} h^{-1}, respectively. Partial pressure of arterial (sciatic) oxygen, Pa_{O_2}, normally about 12–13 kPa, remained unchanged by dehydration, but Pa_{CO_2} increased about 250% from 0.93±0.27 up to 2.27±0.93 kPa. The fall in $D_{Skin}C^{18}O$ during dehydration presumably results at least in part from decreased cutaneous blood flow, possibly in an attempt to reduce the transcutaneous water loss that would otherwise result during dehydrating conditions. Concurrently, cutaneous $\dot{M}_{CO_2}$ is maintained under dehyrdating conditions by a greatly increased Pa_{CO_2} diffusion gradient across the skin. Thus, *Bufo woodhousei* appears able to restrict cutaneous blood flow without compromising vital cutaneous CO_2 loss.

Key words: skin gas exchange, blood flow, dehydration.

Introduction

Cutaneous gas exchange in amphibians, which accounts for approximately two thirds of total CO_2 excretion as well as significant O_2 uptake, occurs across a well-vascularized, relatively thin and lightly keratinized epidermis (see Feder and Burggren, 1985; Boutilier et al., 1992; Shoemaker et al., 1992, for reviews). While cutaneous gas exchange *per se* is relatively well understood in amphibians, the interplay between cutaneous gas exchange and transcutaneous water fluxes remains enigmatic. From a physico–chemical perspective, quite different sets of forces influence water and gas movements: gas movement in both directions depends on partial pressure gradients; dehydration involves a phase change from liquid to gas as water evaporates from the skin; and rehydration involves osmotic or bulk flow. Thus, one might anticipate complex, interrelated roles for skin perfusion in each of these processes. The role of the amphibian skin as a dynamic rather than merely passive barrier to both water loss and water uptake has been long studied. Adolph (1931) reported that dead, skinned aquatic frogs lost water by evaporation in air at rates similar to a free water surface, suggesting that adjustments in cutaneous blood flow are not likely to play a key role in regulating transcutaneous water loss in amphibians. This position has been advocated by several subsequent studies (see Shoemaker and Nagy, 1977; Shoemaker et al., 1992). However, a more recent study showed that the skin of the terrestrial toad *Bufo marinus* offers a significant resistance to

water loss when compared with a free water surface (Dohm et al., 2001).

Despite the uncertain role of blood perfusion in regulating water loss across terrestrial amphibian skin, it is equally well appreciated that cutaneous perfusion in amphibians, particularly of the ventral patch, is under exquisite hormonal and neural control and that skin blood flow in terrestrial amphibians can show enormous ranges (e.g. Burggren and Moali, 1984; West and Van Vliet, 1992; Malvin, 1993; Rea and Parsons, 2001; Viborg and Rosenkilde, 2004; Viborg and Hillyard, 2004). Such changes in skin perfusion have been presumed to regulate environmental water uptake across the skin (especially the ventral patch) rather than limiting water loss. Yet, a recent study by Viborg and Hillyard (2004) shows no relationship between cutaneous blood flow and water uptake in bufonid toads, nor could these researchers find an effect on blood flow produced by Angiotensin II (A II), in contrast with previous studies by Parsons and Schwartz (1991), Parsons et al. (1993) and Slivkoff and Warburton (2001).

Investigation of transcutaneous water fluxes in toads is further complicated by the skin's highly regional morphological and anatomical characteristics, with the bulk of the skin surface area on the dorsal and lateral surfaces responding quite differently than the ventral 'seat patch' (see Viborg and Hillyard, 2004). Moreover, behavioral modifications may reduce 'functional' skin surface area, especially of the ventral surface. Such changes are certainly an important part of terrestrial amphibians' suite of responses aimed at conserving body water (see Hillyard et al., 1998; Viborg and Rosenkilde, 2001).

Against this backdrop of complex, interrelated and sometimes contradictory findings, it is perhaps of little surprise that few studies have concurrently examined both cutaneous gas exchange, and cutaneous water loss and uptake, in terrestrial toads. Consequently, we have used pharmacological tools to probe the relationships between cutaneous gas exchange, body water loss, cardiovascular performance and cutaneous perfusion in *Bufo woodhousei*, a terrestrial anuran that is widely distributed across North America, including the arid conditions of the Mojave Desert in southwestern United States. Specifically, we test the hypothesis that dehydration elicits physiological and/or behavioral responses that compromise cutaneous gas exchange.

Materials and methods

Toads (*Bufo woodhousei* Girard 1854) were collected from the Flamingo Wash in Las Vegas, Nevada during the spring of 1991 and 1992. Mean body mass (±S.D.) of the 23 toads used in this study was 48.2±20.85 g. Animals were housed at 25°C in a large glass aquarium, with free access to water and sandy substrate. All experiments were carried out at 23–25°C.

All measurements described below were performed on undisturbed toads handled carefully to prevent bladder venting, thus allowing them voluntarily to carry an undetermined volume of bladder water according to their physiological and behavioral status.

Blood flow and water loss

Toads were anesthetized (0.5% MS 222 buffered to pH 7.6; Sigma Co., St Louis, MO, USA) and then chronically implanted with a sciatic artery catheter, through which blood pressure could be monitored and drugs injected following recovery from surgery and anesthesia.

After surgery, each toad was placed on wet paper toweling in a tared container (volume ~1 l) through which humidified air was passed at a rate of $1\,l\,min^{-1}$. The catheter was connected to a NARCO pressure transducer and bridge amplifier for recording of arterial blood pressure and heart rate. After a 24 h recovery period, the paper toweling was gently removed from the toad's container. The fully hydrated toads were then weighed by measuring the weight of the toad in the tared container. Care was taken to not disturb the toad during weighing, which could have resulted in bladder venting, but bladder volume was otherwise not controlled for (i.e. 'body weight' in all measurements included an indeterminant bladder weight). Toads were then weighed again at hourly intervals during a period of continual exposure to a stream of dehydrating air (R.H.=0%) until each toad reached ~80% of its hydrated weight (8–12 h). Dehydration to 80% of control body mass was completely reversible without ill effect in these toads. Wet paper towels were then placed back into the containers, allowing the dehydrated toads to rehydrate fully. Body mass was weighed every 30 min during rehydration, since rehydration occurred at a more rapid rate than dehydration. Although specific behaviors were not recorded, each toad was free to move about the chamber and adjust body posture.

On the first day a dehydration run under control conditions was conducted for each toad. To mimic the small blood volume increase caused by drug injections during subsequent dehydration runs, the control run was conducted following injection of a 200 µl volume of saline injected as a sham. After a 24 h recovery period in hydrating conditions, the experiment was repeated on the second day using pharmacological stimulants of cutaneous blood flow. Each toad was given an intra-arterial dose of phentolamine ($5\,mg\,kg^{-1}$; Sigma), a powerful α-blocker leading to vasodilation, and the entire experiment repeated as for the control run. On the third day, each toad was given an intra-arterial dose of isoproterenol ($2\,mg\,kg^{-1}$; Sigma), a powerful cardiac β-stimulant, and the entire experiment repeated a third time. Both drugs were injected in a 200 µl carrier volume of saline that, like the control saline injection, would have a negligible effect on blood volume during the course of the experiment.

Blood pressure and heart rate was measured at hour 1, 3 and 5 h after injection of saline, phentolamine or isoproterenol. Preliminary experiments indicated that the cardiovascular effects of both drugs were in force for approximately 16–18 h, waning quickly thereafter until the return to control values within 24 h of treatment.

Cutaneous carbon dioxide elimination and carbon monoxide diffusing capacity

A face mask for each toad was constructed by the method of Glass et al. (1978). Toads were anesthetized (0.5% MS222) prior to making the plaster impression of their head. Animals were allowed to recover from anesthesia for several days, during which time they had free access to water.

Fully hydrated toads were then weighed and their customized face masks secured in place with cyanoacrylate glue (3M, St Paul, MN, USA). Each toad was placed into a gas-tight glass respirometer (volume ~1 l). The face mask was attached to a tube vented to atmosphere through a port in the wall of the respirometer. This arrangement effectively separated pulmonary gas exchange (with the air from outside the respirometer) from cutaneous gas exchange (with the gas inside the respirometer). A 20 ml mixture of 8.7% He, 26.0% O_2, 9.3% CO, 3.7% $C^{18}O$ and balance N_2 was then introduced into the respirometer. We used an isotope of CO, because the mass of CO and N_2 are identical and cannot be distinguished by a quadrupole mass spectrometer (see below). The $C^{18}O$ used to produce the injected gas mixture came in the form of 28% $C^{18}O$ balanced with CO, accounting for the presence of non-isotopic CO in the injected mixture. After injection of the gas mixture into the respirometer, the gas in the respirometer was fully mixed using alternating filling and emptying of the two glass syringes attached to the lid. Gas was sampled from the respirometer for 30 s every 30 min and analyzed with a quadrupole mass spectrometer (Mediflex V.G. Instruments; Beverly, MA, USA) that had a sample flow rate of 1 ml min^{-1}. The decrease in volume of the system due to gas sampling was accommodated by allowing a decrease in volume of one of the syringes. Consequently, the respirometer remained at atmospheric pressure throughout the experiments. The respirometer volume was determined from the He dilution. Cutaneous CO_2 excretion was calculated from the rate of accumulation of CO_2 into the respirometer. Cutaneous diffusing capacity and O_2 consumption were calculated from the rate of disappearance of $C^{18}O$ and O_2 from the respirometer. Measurements were made every 30 min for a total of three measurements per toad and a mean value was computed. After 90 min, toads were removed from the respirometer. They were immediately placed in a dry air stream to begin a period of dehydration to 80% of hydrated weight. The toads were then returned to their respirometer and the measurements were repeated as described above. This technique determined the integrated gas fluxes across entire exposed surface area, rather than fluxes across specific regions of the skin (e.g. dorsal surface, ventral seat patch).

Blood gas determinations

A separate group of six toads were anesthetized and their sciatic artery occlusively cannulated with PE 50 tubing filled with heparinized saline. After a 24 h recovery period in hydrating conditions, a 300 µl blood sample was then drawn from each animal. Arterial $P_{a_{O_2}}$ and $P_{a_{CO_2}}$ was measured with a Cameron Blood Gas Cell equipped with CO_2 and O_2 electrodes (Microelectrodes; Bedford, NH, USA) whose input was displayed on a Radiometer PHM 73 meter (Radiometer, Copenhagen, Denmark). Electrodes were calibrated with precision gas mixtures. The hematocrit was also determined. Toads were then dehydrated to 75% body weight and blood gas and hematocrit measurements were repeated.

Data analysis

Cardiovascular and gas exchange data were tested for treatment effects with an ANOVA followed by paired *t*-tests (each animal served as its own control). The level of significance chosen was $P<0.05$. All values are presented as means ± 1 S.D.

Results

General effects of dehydration

Dehydration towards 75–80% of control body weight elicited a high level of activity in *Bufo woodhousei*. These activities continued until the toads neared dehydration to 80% of control body mass, whereupon activity decreased and the toads assumed a posture that minimized exposed surface area.

Dehydration, cutaneous blood flow and body water loss

Isoproterenol and phentolamine each produced a large and significant increase in heart rate, with isoproterenol having the greatest effect on both parameters (Fig. 1A). Blood pressure was decreased slightly following α-adrenergic blockade with phentolamine, whereas an increase in blood pressure accompanied treatment with isoproterenol (Fig. 1B). The combined increase in heart rate, and systolic and diastolic blood pressure, with no decrease in pulse pressure strongly suggests an increase in blood flow from isoproterenol, as would be predicted from the known actions of this drug. The increase in heart rate and decrease in blood pressure caused by phentolamine is probably a consequence of peripheral vasodilation leading to increased blood flow rather than central cardiovascular adjustments. Importantly, the skin of the toad following either drug treatment showed the very obvious blushing expected from cutaneous capillary recruitment, especially in the pelvic patch area. Thus, although we were unable to measure cutaneous blood flow directly, the observed cardiovascular effects collectively suggest a large increase in cutaneous blood flow.

Control (saline-treated) toads placed in dehydrating conditions required a mean of 10.1±0.7 h to dehydrate to 80% body mass, equivalent to a dehydration rate of 1.98% of body mass h^{-1}. Toads treated with isoproterenol and phentolamine required mean values of only 7.2±0.8 h and 7.4±0.9 h, respectively, reflecting a dehydration rate about 40% faster than in control toads (Table 1). Fig. 2 shows the time course of rehydration and dehydration in each of the conditions. Note that, compared with control toads, the time course of rehydration was also more rapid in the phentolamine-treated and, to a lesser extent, the isoproterenol-treated toads.

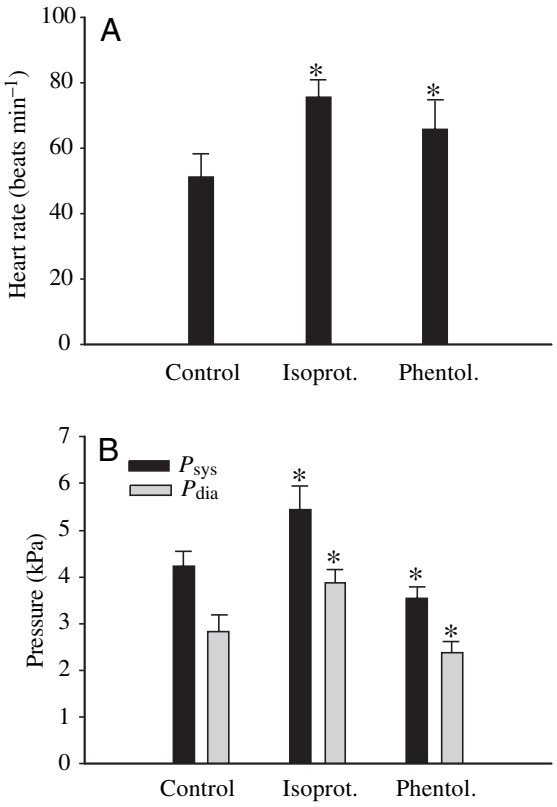

Fig. 1. Effects of the injection of saline, isoproterenol (5 mg kg^{-1}) and phentolamine (2 mg kg^{-1}) on heart rate (A) and systolic and diastolic blood pressure (B) in the toad *Bufo woodhousei*. Values for each toad from hour 1, 3 and 5 following injection were averaged into a single data point for each condition, which were then averaged and plotted as means ± 1 S.D. (*N*=5 toads).

Dehydration effects on gas exchange and diffusion

Following an enforced 20–25% loss of body water, dehydration was accompanied by a 42% decrease in $D_{Skin}C^{18}O$ from 0.49±0.03 μmol g body $mass^{-1}$ h^{-1} kPa in fully hydrated toads down to 0.28±0.09 μmol g^{-1} h^{-1} kPa in dehydrated toads (Table 2). Our hypothesis would thus predict a concomitant decrease in cutaneous gas exchange. However, there were no significant differences (P>0.05) from cutaneous gas exchange values measured in fully hydrated toads, with $\dot{M}_{O_2}$ remaining at about 1.4 μmol g^{-1} h^{-1}, $\dot{M}_{CO_2}$ remaining at about 1.7 μmol g^{-1} h^{-1}, and R remaining between 1.1–1.4 (Table 2).

Dehydration and blood, and blood gases

Dehydration produced a significant increase in hematocrit by nearly 25% (Table 3), presumably due to a decline in blood volume from plasma loss due to dehydration. The Pa_{CO_2} was 0.97 kPa in hydrated toads, rising significantly (P<0.01) by about 2.5 times to 2.27 kPa following dehydration. Pa_{O_2}, with mean values in the range of 12–13 kPa, was as expected more variable than Pa_{CO_2}, but showed no significant change (P>0.05) following hydration.

Discussion

Possible role of activity and posture, during rehydration and dehydration

Responses to dehydration by *Bufo woodhousei* include important behavioral as well as physiological adjustments. The increase in locomotor activity displayed by the toads during the early phases of dehydration is probably a water-searching behavior associated with the stress of dehydration, as discussed by numerous authors (Heatwole and Newby, 1972; Putnam and Hillman, 1977; Brekke et al., 1991; Jorgensen, 1994; Propper and Johnson, 1994; Hillyard et al., 1998; Viborg and Rosenkilde, 2001; Dohm et al., 2001). As dehydration approaches 20–25% of hydrated body weight, postural adjustments are adopted to minimize evaporative water loss through the reduction of exposed surface area. We did not quantify activity in the present experiments. However, it is quite possible that the pharmacologically induced differences in dehydration rates we measured could have resulted not only from some combination of pharmacological effects on cutaneous blood flow and/or skin permeability), but also from behavioral differences leading to differential rates of water loss. Investigating the behavioral effects of these pharmacological agents would be an interesting follow-up to the current experiments.

Mechanisms for altering cutaneous gas permeability

A reduction in overall cutaneous gas permeability, as indicated by a profound decrease in skin CO-diffusing capacity, might comprise an important physiological response

Table 1. *Dehydration and rehydration rates in toads treated with isoproterenol and phentolamine**

	N	Dehydration time, to 80% body mass (h)	Dehydration rate (% h^{-1})	Rehydration time, back to 100% body mass (h)	Rehydration rate (% h^{-1})
Control	11	10.1±0.7	1.98	3.4±0.4	5.88
Isoproterenol	11	7.2±0.8†	2.77†	3.0±0.4	6.67
Phentolamine	11	7.4±0.9†	2.70†	1.6±0.2†	12.5†

*Mean time to dehydration (80% body mass) and rehydration (back to 100% body mass) in control toads and those treated with isoproterenol (5 mg kg^{-1}) and phentolamine (2 mg kg^{-1}).

†Values that are significantly different (P<0.05) from control values. Means ± 1 S.D. are given.

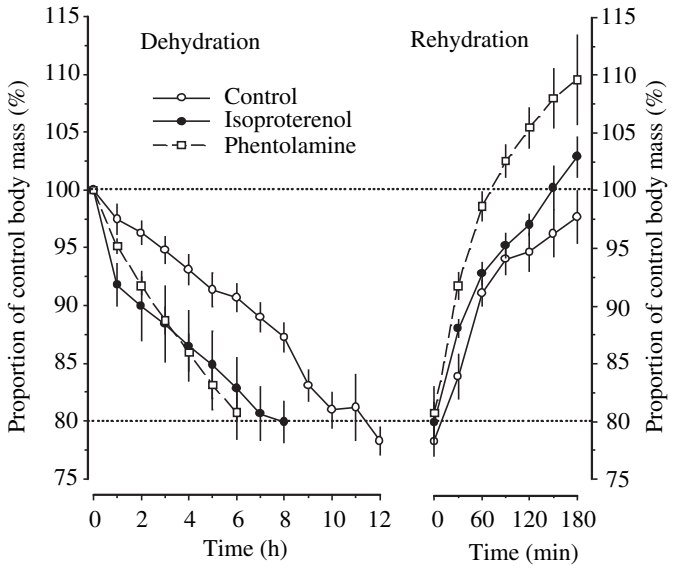

Fig. 2. Time course of dehydration and rehydration rates in control toads (*Bufo woodhousei*) and those treated with isoproterenol or phentolamine. Means ± 1 S.D. are plotted. Note the time-scale difference between the two plots, which reveals a much higher rate of rehydration than dehydration (see also Table 1). *N*=11 toads for each of the three treatments.

to minimize evaporative water loss. Although a prevailing view originally based on frog skin holds that blood flow to skin of amphibians has no effect on skin water loss (e.g. Adolph, 1931), most toads have a highly sculptured skin that potentially presents a significantly greater surface area than the relatively flat skin of aquatic frogs. Toads also tend to have a smaller surface area to volume ratio. These anatomical differences between toads and frogs raise the possibility that active control of cutaneous perfusion might have effects on gas exchange and water loss that might not be anticipated from earlier studies. For example, toads may be able to modify the physiological (as opposed to anatomical) surface area of the skin, across

Table 2. *Cutaneous gas exchange rates in hydrated and dehydrated toads**

Variable	Hydrated	Dehydrated to 80%
N	6	6
Hydrated body mass (g)	50.4±43.2	40.4±34.9†
$D_{Skin}C^{18}O$ (μmol g^{-1} h^{-1} kPa)	0.49±0.03	0.28±0.09†
$\dot{M}_{O_2}$ (μmol g^{-1} h^{-1})	1.43±0.47	1.48±0.15
$\dot{M}_{CO_2}$ (μmol g^{-1} h^{-1})	1.75±0.85	1.72±0.04
R_{Skin}	1.36±0.56	1.13±0.08

*Comparison of cutaneous carbon monoxide diffusion capacity ($D_{Skin}C^{18}O$), cutaneous oxygen uptake ($\dot{M}_{O_2}$), cutaneous carbon dioxide elimination ($\dot{M}_{CO_2}$), and cutaneous gas exchange ratio (R_{Skin}, $\dot{M}_{CO_2}$:$\dot{M}_{O_2}$) between hydrated toads and toads dehydrated to 80% of their hydrated body mass at 23–25°C.

†Dehydrated values that are significantly different (P<0.05) from hydrated values. Means ±1 S.D. are given.

Table 3. *Levels of hematocrit, and arterial partial pressures of CO_2 and O_2, in hydrated and dehydrated toads**

Variable	Hydrated	Dehydrated to 80%
N	6	6
Hydrated body mass (μmol g^{-1} h^{-1} kPa)	76.2±12.6	57.3±11.9†
Hematocrit (%)	30.4±5.3	41.8±8.5†
Pa_{O_2} (kPa)	13.3±1.8	12.3±2.9
Pa_{CO_2} (kPa)	1.0±0.3	2.3±0.9†

*Comparison of hematocrit, and arterial Pa_{CO_2} and Pa_{O_2}, in hydrated toads and toads dehydrated to 75% of their hydrated body mass at 23–25°C.

†Dehydrated values that are significantly different (P<0.05) from hydrated values. Means ±1 S.D. are given.

which not only gas exchange but also water fluxes could occur, by reduced capillary recruitment. Amphibians facing dehydration can alter skin gas exchange by decreasing the number of perfused skin capillaries (e.g. Brown, 1972; Burggren and Moalli, 1984; Malvin and Hlastala, 1989; Slivkoff and Warburton, 2001). Indeed, Malvin et al. (1991) showed that, in *Bufo woodhousei* dehydrated to 75% of hydrated body weight, blood cell flux (an index of blood flow) through the capillaries of the pelvic patch decreased by more than 80%. At the same time, red blood cell velocity fell by more than 75%. Although the responses are more profound in the seat patch, similar changes specifically attributable to decreased capillary recruitment also occur over the dorsal surface of *Bufo woodhousei*, (W.W.B. and G. M. Malvin, unpublished).

Collectively, our data implicate regulated changes in cutaneous blood flow with dehyration/hydration in toads. We conclude that a decrease in capillary blood flow, probably combined with capillary decruitment contributes to, if not causes, the observed decrease in $D_{Skin}C^{18}O$ during dehydration in *Bufo woodhousei*. Such decreases in cutaneous blood flow reduce the ‘functional’ or ‘physiological’ surface area of the skin – not only for water exchange but also for gas exchange (see Burggren and Moalli, 1984; Malvin, 1988).

It is important to indicate that while a linkage between cutaneous blood flow and cutaneous gas exchange appears likely, the link between cutaneous blood flow and transcutaneous water flux remains ambiguous. Recent measurements of cutaneous blood flow in *Bufo alvarius* and *Bufo marinus* by Viborg and Hillyard (2004) reveal no significant correlation between ventral skin blood flow and cutaneous water uptake. Viborg and Rosenkilde (2004) have demonstrated that, during rehydration in toads, arginine vasotocin (AVT) stimulates an inward water flux across skin that is independent of changes in skin blood flow. Recently Hasegawa et al. (2003) localized aquaporins in the entire plasma membrane of the granular cells beneath the outermost layer of skin in the pelvic patch of the frog *Hyla japonica*. These aquaporins were upregulated in response to vasotocin,

suggesting a mechanism independent of blood flow that could account for the potent endocrine control of water permeability at important sites for water flux in amphibians. Clearly, additional experiments are required to examine potential antagonistic and synergistic interactions between cutaneous blood flow and hormonally induced changes in skin permeability.

Rehydration and cutaneous blood flow

Rehydration experiments in *B. woodhousei* revealed several interesting features. Whereas dehydration involves a phase change from liquid to gas associated with evaporation from the skin, rehydration involves osmotic or bulk flow, so different rates of dehydration and rehydration might be anticipated. Indeed, evident in Table 1 and Fig. 2 are much greater rates of rehydration than dehydration for all three experimental conditions. Toads exposed to phentolamine showed an 'overshoot' during rehydration, quickly increasing their body mass by 10% over control values. One possible explanation is that the toads in this particular group were somehow not fully hydrated at the start of the experiment. However, given that all toads were treated identically, and that all toads were kept in hydrating conditions, it is highly unlikely that the stimulation by phentolamine of fluid uptake can be accounted for by different initial states of hydration. Alternative mechanisms are manifold. For example, this rapid rehydration and extra fluid accumulation could relate to an increase in skin perfusion. Increased skin blood flow with relatively high osmolality blood created by dehydration would maintain a steep osmotic gradient for inward water flux across the skin, at least until full normal rehydration levels had been reached. However, as noted earlier, Viborg and Hillyard (2004) failed to find a relationship between ventral seat patch perfusion and water uptake. Another possible mechanism for enhanced water uptake is that phentolamine increased skin water permeability independent of skin blood flow changes, perhaps through water channel insertion as discussed above.

Respiratory implications of reduced cutaneous gas permeability

A reduction in skin water permeability that accompanies a reduction in skin permeability to gases would permit an anuran like *Bufo woodhousei*, whose range extends into relatively (xeric) dry habitats, to spend more time foraging away from water. By reducing skin water permeability and rates of water loss, body water can be conserved and dehydration slowed when water availability is reduced. However, reduced skin permeability also impacts cutaneous gas exchange, a major route in amphibians for oxygen uptake and carbon dioxide elimination (for reviews see Feder and Burggren, 1985, 1992).

Why does carbon dioxide elimination and oxygen uptake across the skin not decline equally precipitously in dehydrated toads? The dramatic increase in $P\mathrm{a}_{\mathrm{CO_2}}$ after 20% dehydration provides a substantially increased gradient for gas exchange across the skin. Since cutaneous gas exchange is traditionally viewed as having a large diffusion limitation (see Feder and Burggren, 1985; Piiper, 1988), a large increase in the $P_{\mathrm{CO_2}}$ gradient across the skin could easily compensate for the documented decrease in the skin's gas diffusing capacity. Additionally, the measured increase in blood $P\mathrm{a}_{\mathrm{CO_2}}$ during dehydration may well have stimulated lung ventilation (see Smatresk and Smits, 1991; Branco et al., 1993; Wang et al., 2004). While only about 1/3 of CO_2 elimination normally occurs via the lungs in bufonids, the enhanced CO_2 partial pressure gradient across the pulmonary membranes under dehydration conditions would probably act in concert with the enhanced $P_{\mathrm{CO_2}}$ gradient across the skin, with both phenomena ensuring ongoing CO_2 elimination despite reduce skin permeability to this gas.

The oxygen partial pressure gradient between arterial blood and air covering the skin did not increase during dehydration, unlike the CO_2 gradient. This apparent inconsistency can be explained by the fact that sciatic blood may not provide an accurate measure of cutaneous arterial $P\mathrm{a}_{\mathrm{O_2}}$, for two reasons. First, intracardiac and/or central vascular shunting may direct more venous blood to the pulmocutaneous artery than to the sciatic artery (see Burggren, 1988; Hedrick et al., 1999; Gamperl et al., 1999; Anderson, 2003). Such shunts could easily result in a $P\mathrm{a}_{\mathrm{O_2}}$ lower in the cutaneous artery, serving large numbers of capillaries over major regions of the trunk, than in the sciatic artery, which provides blood to a relatively small number of skin capillaries on the legs (Moalli et al., 1980). Under this scenario, toads could maintain overall cutaneous O_2 uptake even with no change in sciatic $P\mathrm{a}_{\mathrm{O_2}}$. A second reason why sciatic $P\mathrm{a}_{\mathrm{O_2}}$ may not reflect the $P\mathrm{a}_{\mathrm{O_2}}$ of blood entering the skin capillaries stems from intermittent lung ventilation. Discontinuous patterns of air breathing produce large (5–18 kPa) fluctuations in $P\mathrm{a}_{\mathrm{O_2}}$ in amphibians (see Feder and Burggren, 1992). This makes $P\mathrm{a}_{\mathrm{O_2}}$ a poor indicator of the magnitude of the gradients between animal and environment. In such a situation $P\mathrm{a}_{\mathrm{CO_2}}$ in the sciatic artery would remain a useful indicator of overall blood CO_2 levels because both arterial–venous differences and fluctuations due to the discontinuous nature of breathing are far smaller for CO_2 than O_2.

The increase in hematocrit in dehydrated toads could also play a role in maintaining gas exchange across the skin, provided that hematocrit increases were not so large as to compromise bulk delivery of blood by the cardiovascular system (see Hillman et al., 1985; Hillman, 1987). For example, with the greatly lengthened capillary residence times evident during dehydration (Malvin et al., 1991), blood could become O_2 saturated long before leaving the capillary. Under these conditions an increased hemoglobin concentration in skin capillary blood would facilitate additional diffusion of oxygen from air to blood by acting as an enhanced O_2 sink to lower the quantity of free oxygen molecules dissolved in solution in plasma and thus lower capillary blood $P\mathrm{a}_{\mathrm{O_2}}$. Increased hematocrit could also facilitate CO_2 excretion by providing more intracellular carbonic anhydrase per unit volume of blood to cycle CO_2 held in plasma bicarbonate, although it is not clear whether carbonic anhydrase is indeed rate limiting under any

circumstances. Additionally, increased hematocrit (and the attendant increased hemoglobin concentration) would provided greater hemoglobin buffering of the increased [H^+] that might attend the elevated blood Pa_{CO_2} levels. Given the large extent of water loss associated with the measured 25% increase in hematocrit during dehydration, the most parsimonious explanation for the hematocrit increase is a simple decrease in blood volume through plasma loss. A release of red blood cells sequestered in the spleen might also contribute to the elevated hematocrit, though this process is poorly understood in amphibians.

Finally, we did not measure bladder water volume nor interfere with its retention, as one of our goals was to examine the effects of adrenergic drugs on heart rate and blood flow independent of any physiological or behavioral defenses against dehydration that could be mounted. Yet, the apparent similarity of the proportional increase in hematocrit and the proportional decrease in body water during dehydration suggests that these toads were either not using any retained bladder water to defend body tissue dehyration, or such use of bladder water use was ineffective.

Conclusions

Bufo woodhousei reduces skin gas-diffusing capacity during dehydration. Although the mechanism is uncertain, there is a related reduction in transcutaneous water loss that correlates with a reduction in skin blood flow. The physiological conflict between the need to continue cutaneous gas exchange while potentially lowering evaporative water loss through reduced skin flow in *Bufo woodhousei* appears to be circumvented by compensatory increases in gas exchange gradients and possibly fortuitous changes in hematocrit. So long as *Bufo woodhousei* can tolerate the acid–base and other implications of a considerably elevated blood and tissue P_{CO_2}, then it can simultaneously achieve both reduced water loss and maintained CO_2 elimination across the skin during dehydrating conditions. Such circumvention of perceived physiological conflicts may in fact be more common than previously supposed, but requires multi-faceted experiments that are specifically designed to test such hypotheses.

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