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Osmotic Effects on Mitochondria from Two Species of Amphibian, *Bufo marinus* and *Rana catesbeiana*

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Abstract

The effects of ionic and/or osmotic change on skeletal muscle mitochondrial performance were investigated. Two substrates, pyruvate and glutamate, and variation in osmotic pressure from 205 to 360 mosm in KCl or mannitol/sucrose media had no effect on maximal respiratory rate (state 3) or coupling (respiratory control ratio) in either species. Over an equivalent range of osmolalities associated with dehydration, organismic maximal O₂-consumption rates are severely diminished with increasing osmolalities. The data do not support a mitochondrial limit to organismic O₂ consumption under dehydration. There were interspecies differences in state 3 respiration and coupling that were similar to differences noted in mitochondria isolated from fish red and white muscle, with toad mitochondria behaving more like red muscle and frog mitochondria behaving more like white muscle.

Introduction

There are two hypotheses for the primary limitation to maximal rates of O₂ consumption ($\dot{V}O_2$ max) in anuran amphibians. The first is that $\dot{V}O_2$ max is limited by the mitochondrial capacity of skeletal muscle. Data supporting this view include (1) the correlation between interspecific differences in $\dot{V}O_2$ max and differences in citrate synthase activity (Putnam and Bennett 1983), (2) the enhancement by training of endurance and citrate synthase activity (Cummings 1979), and (3) a high mitochondrial density in the skeletal musculature associated with calling in species with high $\dot{V}O_2$ max during calling (Taigen, Wells, and Marsh 1985; Marsh and Taigen 1987). The second hypothesis is that systemic O₂ transport limits $\dot{V}O_2$ max. Data supporting this hypothesis are that (1) interspecific differences in $\dot{V}O_2$ max correlate

with differences in ventricular mass and hemoglobin concentration (Hillman 1976) and (2) experimentally induced variation in maximal heart rate, stroke volume, and hemoglobin concentration correlate with changes in $\dot{V}O_2$ max of the animal (Hillman 1980a, 1982; Hillman et al. 1985). Only one study has attempted to simultaneously correlate both mitochondrial and cardiovascular variables with individual variation in $\dot{V}O_2$ max and found correlation only with the cardiovascular variables (Walsberg, Lea, and Hillman 1986).

Maximal aerobic capacity in a variety of anurans has been shown to decline during dehydration (Hillman 1978; Pough et al. 1983; Gatten 1987; Hillman 1987), and that $\dot{V}O_2$ max declines in proportion to maximal blood flow rate during dehydration (Hillman 1987). Terrestrial species show greater resistance to this effect. If the mitochondrial limit hypothesis is correct, mitochondrial function should decline with increased osmolality and the decline should be less in more dehydration-tolerant species, with the assumption that the increase in osmotic and ionic strength associated with tissue water loss is the effective variable.

The specific aim of this study is to determine the effect of changing ionic strength and/or osmotic concentration on the performance of isolated mitochondria from two amphibian species that differ in their tolerance to dehydration and hyperosmolality. This will provide a test of the mitochondrial limitation to $\dot{V}O_2$ max hypothesis under dehydration as well as providing data on the effects of hyperosmolality on mitochondrial function in terrestrial vertebrate species.

Material and Methods

Animals

Bufo marinus ($\bar{X}$ mass = 420 g) and *Rana catesbeiana* ($\bar{X}$ mass = 290 g) were purchased from commercial suppliers. Both species were given access to water, and experiments were conducted within 3 wk of the species' arrival in the laboratory.

Isolation of Mitochondria

Animals were doubly pithed, and thigh musculature was rapidly excised, cut with scissors, and weighed in 10 mL ice-cold isolation medium in a beaker. Isolation medium contained 0.170 M mannitol, 0.055 M sucrose, 0.005 M ethyleneglycol-bis- β aminoethyl ether N, N, N'-tetraacetic acid (EGTA), and 0.02 M Tris-HCl pH 7.2 (at room temperature), in twice-glass-distilled water. To this were added 0.5% bovine serum albumin (BSA) with

less than 0.005% fatty acids and 50 units/mL heparin. The final osmotic pressure, 270 mosm (270 mosm/kg H₂O), was determined with a vapor pressure osmometer (Wescor Model 523A). The muscle was minced with scissors, homogenized at low speed (1,000 rpm for about 30 s), and then forced through a steel plate with holes 0.8 mm in diameter by a threaded piston in a specially made tissue press. The homogenate was washed into a Potter-type glass homogenizer and ground with two pestles of increasing diameter (two complete strokes each) at slow speed (about 200 rpm). The Teflon pestles had a clearance of about 0.15 mm and 0.05 mm (Tri-R Instruments). The homogenate was spun at 755 g for 5 min, the supernatant centrifuged at 9,800 g for 10 min, the pellet surface was rinsed with isolation medium, and the pellet resuspended and spun at 9,800 g for 7 min. The final pellet was resuspended in isolation medium (100 μ L medium per gram muscle) lacking BSA and heparin. A 50- μ L aliquot was frozen for protein determination (Lowry et al. 1951).

Measurement of Respiration

Oxygen uptake was measured at 20°C in a Yellow Springs Oxygen Monitor model 53 with a Clark-type electrode (3 mL per vial). Each vial contained 50 μ L of mitochondrial suspension, 30 μ L of 1.0 M glutamic acid, and 2.9 mL of O₂ uptake medium. All O₂ uptake media contained a basal medium: 10 mM KH₂PO₄, 90 mM KCl, 5 mM malate, and 10 mM Tris buffer, adjusted to pH 7.4 at room temperature. Each series of experiments included determination of O₂ uptake and respiratory control ratios in this basal medium plus either 0.017 M mannitol/sucrose or 0.011 M KCl (osmotic pressure 205 mosm). In the rest of each series, the basal medium was supplemented with mannitol and sucrose (3:1 molar ratio) or KCl to obtain solutions of increasing osmotic pressure. The osmotic pressures of all stock solutions, the basal media, and the other media were measured with a vapor pressure osmometer. The osmotic pressures given for each medium do not include the mitochondrial suspension, the glutamate or pyruvate substrate, or the ADP. Respiratory control ratios (RCR) were measured as the ratio of the rate of O₂ uptake in the presence of ADP (after ADP solution was injected into vials) to the rate after the exhaustion of the ADP, that is, the ratio state 3:state 4 (Chance and Williams 1956; Estabrook 1967). The O₂ content of the media was assumed to be 281 μ M as previously determined (Lea and Hillman 1990).

All statistical comparisons were made with ANOVA with Scheffe's tests for multiple comparisons, unless specifically noted. A $P < 0.05$ was considered significant.

Results

There was no effect ($P > 0.05$) of increasing the osmotic concentration from 205 mosm to 360 mosm on either state 3 respiration or respiratory control ratios in either species in all media and substrates (figs. 1, 2). There were no effects of media (mannitol/sucrose or KCl) or substrate (pyruvate or succinate) on states 3 or 4 respiration or RCR in either species. The only significant effect noted was on state 4 respiration for *Bufo marinus* as osmotic concentration increased in KCl uptake medium with pyruvate as a substrate ($P < 0.05$), but this change was small enough that it did not significantly influence RCRs.

Fig. 1. The effect of osmotic concentration on state 3 respiration (upper data, top trace), state 4 respiration (lower data), and RCRs in skeletal muscle mitochondria from *Bufo marinus*. Triangles = KCl medium (n = 3), circles = mannitol/sucrose medium (n = 6), filled symbols = glutamate substrate, unfilled symbols = pyruvate substrate. Values are means; vertical lines = 1 SE.

Fig. 2. The effect of osmotic concentration on state 3 respiration, state 4 respiration, and RCRs in skeletal muscle mitochondria from *Rana catesbeiana*. For details see fig. 1.

Since there were no significant effects of osmotic concentration, media, or substrate in either species for the parameters, measured data were pooled (except state 4, KCl-pyruvate in *B. marinus*) to make interspecific comparisons (table 1). *Bufo marinus* had significantly greater rates of state 3 respiration ($P < 0.001$), lower rates of state 4 respiration ($P < 0.001$), and hence higher RCRs ($P < 0.001$) than *Rana catesbeiana*.

Discussion

Amphibians can experience wide fluctuations in their body fluid concentrations as a consequence of high rates of cutaneous evaporative water loss and their inability to produce a urine hyperosmotic to their own body fluids.

TABLE 1
Summary mitochondrial values as mean $\pm$ SE

	<i>Bufo marinus</i>	<i>Rana catesbeiana</i>
State 3 respiration (nmol O ₂ /mg $\cdot$ min)	40.9 $\pm$ 1.1 (55)	31.3 $\pm$ 1.5 (64)
State 4 respiration (nmol O ₂ /mg $\cdot$ min)	4.5 $\pm$.2 (40)	5.8 $\pm$.3 (64)
RCR	9.1 $\pm$.5 (55)	6.0 $\pm$.4 (64)

Note. Sample sizes are in parentheses. The two species columns differed significantly ($P < 0.001$) for all three parameters.

Tolerance to hyperosmolality seems to be an important adaptation in the colonization of terrestrial habitats (Hillman 1980*b*) and for some species colonization of hypersaline habitats (Gordon, Schmidt-Nielsen, and Kelly 1961; Gordon 1965; Katz 1973; Romsper and McClanahan 1981). Increasing osmotic concentration with KCl would increase both the ionic and osmotic strength of the extramitochondrial milieu, while mannitol/sucrose would simply increase the osmotic concentration. In neither solution was mitochondrial performance influenced, indicating tolerance to increases in osmotic concentration from 205 to 360 mosm in both species. This is a broad tolerance for mitochondria relative to mammals (Holtzman et al. 1978), but it is consistent with broad osmotic tolerances reported for both teleost and elasmobranch fishes (Anderson 1986; Ballantyne and Moon 1986; Lea and Hillman 1990).

The data do not support the hypothesis for a direct mitochondrial limit to aerobic metabolism during dehydration. The aerobic capacity of *Bufo marinus* is more tolerant of dehydration than that of *Rana catesbeiana* (see fig. 3). The mitochondrial performance was not affected, nor did it differ interspecifically over an equivalent range of osmolalities (fig. 3).

Dehydration might not directly influence mitochondrial function but act indirectly by limiting substrate availability. The most plausible substrate for mitochondrial oxidation during burst activity is pyruvate from glycolysis, which has been shown to increase with lactate during activity in *R. catesbeiana* (Quinn and Burggren 1983). Rates of anaerobic conversion of pyruvate to lactate during activity are not influenced by dehydration in either *Rana pipiens* or *Bufo americanus* (Gatten 1987). Therefore, pyruvate availability as a substrate for anaerobic metabolism does not appear to change

Fig. 3. The effect of osmotic concentration on state 3 mitochondrial respiration (filled symbols) and organismic $\dot{V}O_2$ max (unfilled symbols; Hillman 1987) in *Rana catesbeiana* (circles) and *Bufo marinus* (triangles). Osmotic concentrations for organismic data were calculated from the relationship of water loss to extracellular fluid concentration (Schoemaker 1964) and with the assumption of osmotic equality of intracellular and extracellular fluid compartments (Gordon 1965).

during dehydration. Since anaerobically supplied ATP accounts for approximately 70% of the total ATP during activity in *R. pipiens* (Carey 1979), there should be no limitation of pyruvate for aerobic metabolism. There are no data on utilization of fatty acids or amino acids as mitochondrial substrates during maximal activity in amphibians or whether dehydration influences their availability.

The yield of mitochondria was greater in *B. marinus* than *R. catesbeiana* based on our isolation techniques, which is consistent with differences in citrate synthase activity noted in other species of *Bufo* and *Rana* (Putnam and Bennett 1983). It is interesting that mitochondria from *R. catesbeiana* had lower state 3 rates of respiration and were less well coupled than mitochondria from *B. marinus*. In comparisons of mitochondria isolated from carp red and white muscle (Moyes et al. 1989), white muscle mitochondria were more poorly coupled and had lower citrate synthase activity per milligram of mitochondrial protein, although state 3 rates of respiration were equivalent. This may indicate qualitatively different mitochondria in *B. marinus* compared with *R. catesbeiana* aside from quantitative differences.

In conclusion, we have demonstrated that mitochondria isolated from both species are insensitive to osmotic changes that would be associated

with water losses of 30% of initial body mass. Given that organismic aerobic capacity is severely compromised over equivalent degrees of water loss and osmotic increases, we feel the data argue against a mitochondrial limit to organismic aerobic capacity in these species under dehydration. Since systemic blood flow declines in parallel with aerobic capacity in both these species under dehydration (Hillman 1987), we feel a cardiovascular limitation to aerobic capacity is the strongest explanation for the decline in aerobic capacity. The data are also consistent with fundamentally different types of mitochondria in the two species as well as differences in mitochondrial concentration in skeletal muscle.

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Literature Cited

- ANDERSON, P. M. 1986. Effects of urea, trimethylamine oxide and osmolality on respiration and citrulline synthesis by isolated hepatic mitochondria from *Squalus acanthias*. Comp. Biochem. Physiol. 45A:1009–1021.
- BALLANTYNE, J. S., and T. W. MOON. 1986. Solute effects on mitochondria from an elasmobranch (*Raja erinacea*) and a teleost (*Pseudopleuronectes americanus*). J. Exp. Zool. 239:319–328.
- CAREY, C. 1979. Aerobic and anaerobic energy expenditure during rest and activity in montane *Bufo b. boreas* and *Rana pipiens*. Oecologia 39:213–228.
- CHANCE, B., and G. R. WILLIAMS. 1956. The respiratory chain and oxidative phosphorylation. Adv. Enzymol. 17:65–135.
- CUMMINGS, J. W. 1979. Physiological and biochemical adaptations to training in *Rana pipiens*. J. Comp. Physiol. 134:345–350.
- ESTABROOK, R. W. 1967. Mitochondrial respiratory control and polarographic measurement of ADP/O ratios. Pages 41–47 in S. P. COLOWICK and N. O. KAPLAN, eds. Methods in enzymology. Vol. 10. Academic Press, New York.
- GATTEN, R. E. 1987. Activity metabolism of anuran amphibians: tolerance to dehydration. Physiol. Zool. 60:576–585.
- GORDON, M. S. 1965. Intracellular osmoregulation in skeletal muscle during salinity adaptation in two species of toads. Biol. Bull. 128:218–229.
- GORDON, M. S., K. SCHMIDT-NIELSEN, and H. M. KELLY. 1961. Osmotic regulation in the crab-eating frog (*Rana cancrivora*). J. Exp. Biol. 38:659–678.

- HILLMAN, S. S. 1976. Cardiovascular correlates of maximal oxygen consumption rates in anuran amphibians. *J. Comp. Physiol.* 109:199–207.
- . 1978. The roles of oxygen delivery and electrolyte levels in the dehydrational death of *Xenopus laevis*. *J. Comp. Physiol.* 128:169–175.
- . 1980a. The effect of anemia on metabolic performance in the frog, *Rana pipiens*. *J. Exp. Zool.* 211:107–111.
- . 1980b. Physiological correlates of differential dehydration tolerance in anuran amphibians. *Copeia* 1980:125–129.
- . 1982. Effects of DL-propranolol on exercise heart rate and maximal rates of oxygen consumption in *Scaphiopus intermontanus*. *Experientia* 38:940–941.
- . 1987. Dehydrational effects on cardiovascular and metabolic capacity in two amphibians. *Physiol. Zool.* 60:608–613.
- HILLMAN, S. S., P. C. WITHERS, M. S. HEDRICK, and P. B. KIMMEL. 1985. The effects of erythrocythemia on blood viscosity, maximal systemic oxygen transport capacity and maximal rates of oxygen consumption in an amphibian. *J. Comp. Physiol.* 155:577–581.
- HOLTZMAN, D., N. LEWISTON, M. M. HERMAN, M. DESAUTEL, E. BREWER, and E. ROBIN. 1978. Effects of osmolar changes on isolated mitochondria of brain and liver. *J. Neurochem.* 30:1409–1419.
- KATZ, N. 1973. Studies on the adaptation of the euryhaline toad *Bufo viridis* to high salinities: oxygen consumption, plasma concentrations and water content of the tissues. *J. Exp. Biol.* 58:785–796.
- LEA, M. S., and S. S. HILLMAN. 1990. Effects of osmolality and solutes on performance of shark heart mitochondria. *J. Exp. Zool.* 255:9–15.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
- MARSH, R. L., and T. L. TAIGEN. 1987. Properties enhancing aerobic capacity of calling muscles in gray tree frogs *Hyla versicolor*. *Am. J. Physiol.* 252:R786–R793.
- MOYES, C. D., L. T. BUCK, P. W. HOCHACHKA, and R. K. SUAREZ. 1989. Oxidative properties of carp red and white muscle. *J. Exp. Biol.* 143:321–331.
- POUGH, F. H., T. L. TAIGEN, M. M. STEWART, and P. F. BROUSSARD. 1983. Behavioral modification of evaporative water loss by a Puerto Rican frog. *Ecology* 64:244–252.
- PUTNAM, R. W., and A. F. BENNETT. 1983. Histochemical, enzymatic, and contractile properties of skeletal muscles of three anuran amphibians. *Am. J. Physiol.* 244:R558–R567.
- QUINN, D., and W. BURGREN. 1983. Lactate production, tissue distribution, and elimination following exhaustive exercise in larval and adult bullfrogs *Rana catesbeiana*. *Physiol. Zool.* 56:597–613.
- ROMSPERT, A. P., and L. L. MCCLEANAHAN. 1981. Osmoregulation of the terrestrial salamander, *Ambystoma tigrinum*, in hypersaline media. *Copeia* 1981:400–405.
- SHOEMAKER, V. H. 1964. The effects of dehydration on electrolyte concentration in a toad, *Bufo marinus*. *Comp. Biochem. Physiol.* 13:261–271.
- TAIGEN, T. L., K. D. WELLS, and R. L. MARSH. 1985. The enzymatic basis for high metabolic rates in calling frogs. *Physiol. Zool.* 58:719–726.
- WALSBERG, G. E., M. S. LEA, and S. S. HILLMAN. 1986. Individual variation in maximum aerobic capacity: cardiovascular and enzymatic correlates in *Rana catesbeiana*. *J. Exp. Zool.* 239:1–5.