

## SHORT COMMUNICATION

### MYOSIN ATPase ACTIVITY OF MUSKRAT, GUINEA PIG AND RAT HEARTS

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*Accepted 19 October 1989*

Musk rats (*Ondatra zibethicus*) are among the most common freshwater diving mammals in North America. They have a well-developed diving response that occurs upon either forced or voluntary diving (Jones *et al.* 1982). This response consists of a decline in heart rate and cardiac output and a redistribution of blood flow primarily to the brain, heart and adrenal gland, with other organs being hypoperfused. Musk rats have large oxygen stores which are in the lower range of those reported for marine mammals (Snyder and Binkley, 1985). In a previous study on the metabolism of isolated muskrat and guinea pig hearts, it was shown that muskrat hearts do not pump as well as guinea pig hearts (McKean *et al.* 1986). This was indicated by the failure of muskrat hearts to develop a cardiac output in excess of that supplying their own coronary blood flow when the isolated hearts were placed in the working mode and were required to pump fluid. It has also been demonstrated that muskrat hearts are less sensitive than guinea pig hearts to the  $\beta$ -adrenergic agonist, isoproterenol (McKean, 1986). In response to a maximal dose of isoproterenol, left ventricular pressure increased from 9.3 to 10.7 kPa in the muskrat heart, whereas it rose from 9.3 to 18.7 kPa in the guinea pig heart (1 mmHg=0.133 kPa). Part of the reason for the smaller pressure rise seen in the muskrat hearts is a lower density of cardiac membrane  $\beta$ -adrenoreceptors compared with guinea pig hearts (McKean, 1988). There might also be a reduced capacity for oxidative metabolism. The concentrations of specific mitochondrial enzymes have not yet been determined in these species but there is a lower concentration of mitochondrial protein in muskrat hearts compared with both guinea pig and rat hearts (McKean, 1987). All these results suggest that the muskrat heart may be limited in its pumping power compared with guinea pig and rat hearts.

There is a correlation between crude ATPase activity of the heart and resting cardiac power in a wide spectrum of vertebrates (hagfish to swine, Driedzic *et al.* 1987). Within a class, small animals have much larger mass-specific cardiac outputs than larger animals. This is manifest in greater ATPase activities and activities of a number of oxidative enzymes such as citrate synthase and cytochrome oxidase.

Key words: muskrat, ATPase, myosin, heart.

The purpose of the present study was to compare the ATPase activities of muskrat, guinea pig and rat heart to determine if the muskrat is an exception to the allometric relationship between enzyme activity and body mass.

Musk rats weighing between 700 and 1200 g were live-trapped in Idaho and Washington and were used within 2 days of capture. Guinea pigs (500–1000 g) and rats (200–450 g) were obtained from licensed animal dealers. Both sexes were used in the study. Animals were anaesthetized with diethyl ether and their hearts were removed. To isolate and measure cardiac myosin-ATPase activity, the atria and right ventricle were dissected free and the remaining left ventricle was placed in 15 vols of an ice-cold solution of 40 mmol l<sup>-1</sup> NaCl and 3 mmol l<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>, pH 7.0 (Klein and Hong, 1986). In some experiments phenylmethylsulphonyl fluoride (0.2 mmol l<sup>-1</sup>) was present in the initial homogenization solution. All procedures were carried out in a 4°C cold room or on ice. The hearts were homogenized with a Brinkman (Westbury, NY) Polytron homogenizer and then centrifuged at 800 g for 10 min. The pellet was washed once in the same buffer and then suspended in 30 vols of a myosin extraction medium containing 100 mmol l<sup>-1</sup> Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 5 mmol l<sup>-1</sup> EGTA, 5 mmol l<sup>-1</sup> dithiothreitol, 5 mmol l<sup>-1</sup> ATP, 5 mmol l<sup>-1</sup> MgCl<sub>2</sub> and 5 µg ml<sup>-1</sup> leupeptin at pH 8.6. Myosin was extracted at 4°C for 1 h and the suspension centrifuged at 100 000 g for 1 h. The supernatant containing the myosin was dialysed for 18 h at 5°C against a solution of 500 mmol l<sup>-1</sup> KCl and 0.5 mmol l<sup>-1</sup> cysteine at pH 7.0. Myosin-ATPase activity was assayed in 1 ml of incubation medium containing 500 mmol l<sup>-1</sup> KCl, 5 mmol l<sup>-1</sup> NaN<sub>3</sub>, 10 mmol l<sup>-1</sup> CaCl<sub>2</sub>, 1 mmol l<sup>-1</sup> ATP and 100 mmol l<sup>-1</sup> glycine at pH 9.1. To initiate the reaction, 100 µg of extract protein was added to the assay mixture. The assay was performed in triplicate and was linear with respect to added protein and to time. Standard assay conditions were 20°C for 5 min. The reaction was terminated by the addition of an equal volume of 10 % trichloroacetic acid. After centrifugation a sample of the supernatant was assayed for the liberated phosphate (Rockstein and Herron, 1965) using the dialysis buffer as the blank. Protein determinations were made using the Bio-Rad (Richmond, CA) protein assay.

The ATPase activity that was measured was either exclusively or mainly myosin-ATPase activity. The evidence for this is as follows. Approximate relative molecular masses of the contents of the myosin extraction medium were determined using HPLC. A TSK 3000 SW analytical column was used with 0.1 mol l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.2 mol l<sup>-1</sup> NaCl solvent, pH 7.4, at 6.5 MPa pressure and a flow rate of 0.7 ml min<sup>-1</sup>. Detection was at 280 nm. The results of HPLC indicated that there was a high (>300 000) relative molecular mass complex in the myosin extracts of all three species. This peak probably represents myosin which has an intact relative molecular mass of 460 000 (Murry *et al.* 1988). Myosin obtained from Sigma Chemical Company, St Louis, MO (M6643) and run on HPLC also had this same peak. There were several small peaks between the relative molecular masses of 20 000 and 67 000. The area of the largest of these peaks was only 6 % of the large putative myosin peak. Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATPase and Na<sup>+</sup>/K<sup>+</sup>-ATPase are pres-

ent in heart muscle and have relative molecular masses between 100 000 and 175 000 (Darnell *et al.* 1986). Compounds in these relative molecular mass ranges were not detected by HPLC. The various HPLC fractions were collected and analysed for protein. Only in the putative myosin peak was protein detected. Very small amounts of protein in other fractions could have escaped detection. However, all or most of the protein in the extraction medium was myosin. Myosin-ATPase activity does not depend on divalent cations being present in the system, whereas the activity of other ATPases does (Darnell *et al.* 1986). ATPase activity in this study did not change when EGTA ( $15 \text{ mmol l}^{-1}$ ) or ouabain ( $0.5 \text{ mmol l}^{-1}$ ) was added to the system, showing that the ATPase activity was myosin-ATPase and not  $\text{Na}^+/\text{K}^+$ -ATPase.

Both the guinea pig and the rat had significantly greater concentrations of extractable protein in the left ventricle compared with the muskrat (Fig. 1). ATPase activity per mg protein was greatest in the rat, which is known to have large amounts of the V1 isoform of myosin (Lompre *et al.* 1981). This isoform has the greatest ATPase activity and fastest velocity of shortening (Pagani and Julian, 1984) of the three ventricular isoforms. The guinea pig heart had the lowest ATPase activity and it is known to have the V3 isoform which has the least ATPase activity and is the slowest contracting. ATPase activity was only slightly higher in the muskrat than in the guinea pig heart. The isoform distribution for muskrat heart is not known. Rat hearts showed over three times the ATPase activity of either muskrat or guinea pig hearts (Fig. 2). However, muskrat hearts had a greater total ATPase activity than guinea pig hearts. Thus, the observation that

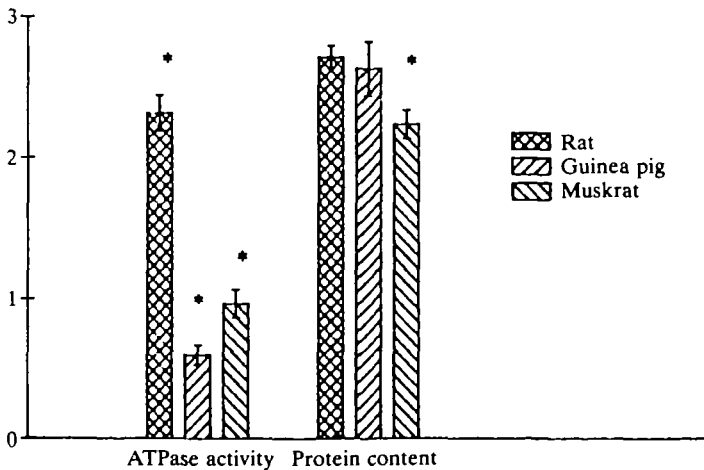


Fig. 1. ATPase activity (left) and protein content of left ventricle (right) in rat ( $N=12$ ), guinea pig ( $N=7$ ) and muskrat ( $N=5$ ) heart. ATPase activity is given as  $\mu\text{mol phosphate mg protein}^{-1} 5 \text{ min}^{-1}$ . Protein content is given as mg protein per 100 mg of left ventricle. \* signifies that the underlying group differs significantly from the other groups using the Student's *t*-test with  $P < 0.05$ . Data are presented as the mean  $\pm$  one standard deviation.

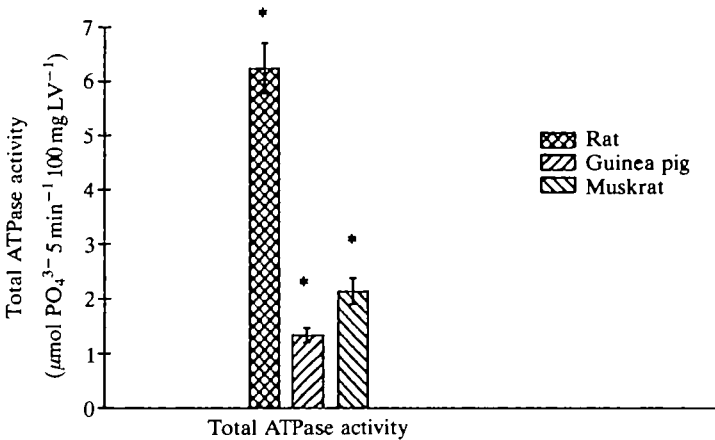


Fig. 2. Total ATPase activity in rat, guinea pig and muskrat left ventricle (LV). *N* and \* are the same as in Fig. 1.

isolated muskrat hearts do not pump as well as guinea pig hearts cannot be explained on the basis of ATPase activity.

Citrate synthase and cytochrome oxidase activities of vertebrate hearts have been found to be positively correlated with resting cardiac power (Driedzic *et al.* 1987). Cytochrome oxidase activity was 31 % greater in the guinea pig heart than in the muskrat heart, but the concentrations of cytochromes *aa*<sub>3</sub>, *b* and *c*<sub>1</sub> are about the same in the hearts of the two species (McKean *et al.* 1986). Total citrate synthase activity was determined in heart muscle homogenate of five rats, four guinea pigs and five muskrats using the method of Shepard and Garland (1969). The rat heart had the highest activity of citrate synthase ( $103.6 \pm 18.3$  units g left ventricle<sup>-1</sup>) followed by the muskrat ( $82.1 \pm 11.0$  units g<sup>-1</sup>) and the guinea pig ( $67.4 \pm 14.4$  units g<sup>-1</sup>). This study shows that mitochondrial yields measured by citrate synthase activity per gram of left ventricle do not agree with previously reported yields based on mitochondrial protein per gram of left ventricle (McKean, 1987). This is probably due to differences in tissue friability among species. This study does show, however, that the rat heart has high levels of ATPase and citrate synthase activity compared with either the muskrat or the guinea pig heart. Rats are smaller animals than either muskrats or guinea pigs and have faster heart rates and larger mass-specific cardiac power. Muskrats and guinea pigs are similar-sized animals with similar resting heart rates. Even though the muskrat is a diving mammal with a heart adapted for hypoxia (McKean, 1984), its heart has similar activities of myosin-ATPase and oxidative enzymes to the guinea pig heart. The conclusions of this study are: (1) the inferior pumping ability of the muskrat heart compared with the guinea pig heart cannot be explained by differences in ATPase activities; and (2) ATPase activities in the rat, muskrat and guinea pig heart scale according to the body mass of the species.

I thank Margaret Dibble and Madison Powell for technical assistance and Rolf

Ingermann for valuable discussion. This study was supported by a grant-in-aid from the American Heart Association of Idaho.

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