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DIFFERENTIAL EFFECTS OF ULTRASONICATION ON THE MYOFIBRILLAR AND MITOCHONDRIAL ATPase ACTIVITY OF "SLOW" AND "FAST" MUSCLES IN BIRDS AND MAMMALS

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STUDIES on myosin from fast (white) and slow (red) muscles of the same species have revealed strong evidence for their marked structural and physiological differences[1]. It has been reported that myofibrillar-ATPase activity increases on ultrasonication of the skeletal muscle myofibrils[2]. There are no particular data available which indicate the differential pattern of the effect of ultrasonication on myofibrillar and mitochondrial ATPase activity in different types of muscles in different animal species. Hence, in the present study, experiments were carried out on the heart, pectoralis and gastrocnemius muscles of the common blue rock pigeon (*Columba livia*), and on the heart, pectoralis and soleus muscles of the adult albino rat (*Rattus rattus*). The heart and soleus muscles represent the "slow" type and pectoralis and gastrocnemius the "fast" type. The differential effects of ultrasonication on both types of muscles in the two species are compared and their implications discussed.

The individual muscles were carefully dissected from the animals and stored in beakers, chilled in ice. The myofibrils were obtained basically by the method of Perry and Grey[3], and the mitochondrial fraction was isolated from the supernatant fraction by using the conventional centrifugation method. The myofibrillar and mitochondrial–ATPase activities were measured with and without ultrasonic treatment, the details regarding the ultrasonication method followed are described earlier[2]. In both (myofibrillar and mitochondrial) fractions Ca^{++} as well as Mg^{++}-activated–ATPase assays were carried out. ATPase assasy were carried out at pH 7·5 and 37° C. In the Ca^{++}–ATPase assay incubation medium, 40 mM Tris-HCl, 40 mM KCl, 10 mM $CaCl_2$, 0·2 ml of myofibrils/mitochondria and 3 mM of ATP were used, whereas in Mg^{++}–ATPase assay the concentration of $CaCl_2$ was 0·2 mM and in addition to this 3 mM of $MgSO_4$ solution was used, the rest of the reactants were the same in both the assays. The final volume of the reactants in both assays (Ca^{++} and Mg^{++}-activated) was 1·5 ml. The reaction was started with the addition of ATP and was stopped by the addition of 1·5 ml of 10% trichloroacetic acid. The amount of Pi liberated and protein content present were measured by the methods of Rockstein and Herron[4] and Gornall *et al.*[5] respectively. All the values of specific-ATPase activity were expressed as μ moles of Pi liberated/mg protein/min.

On the basis of results, it is clearly indicated (Table I) that myofibrillar–ATPase activity of skeletal muscles increases on ultrasonication, which is consistent with our earlier work[2]. This table also shows that the effect of ultrasonication is well marked in "fast" muscles (pectoralis major and gastrocnemius) as compared with that of the "slow" muscle (heart) in pigeon. There is almost no effect of ultrasonication on the mitochondrial–ATPase activity of the three muscles studied.

Table II shows the same pattern of results in rat muscles. Here the change in myofibrillar–ATPase activity of "fast" muscle (pectoralis major) is much more as compared to the change in "slow" muscles (heart and soleus) after ultrasonic irradiation.

TABLE I

Differential effects of ultrasonication on the myofibrillar and mitochondrial–ATPase activities of different skeletal muscles of pigeon

ATPase activity expressed as μ moles of Pi liherated/mg protein/min

Muscle	Myofibril/ mitochondria	Specific–ATPase activity					
		Myofibrillar–ATPase				Mitochondrial–ATPase	
		Ca^{++}– activated	P*	Mg^{++}– activated	P*	Ca^{++}– activated	Mg^{++}– activated
Heart	Normal	0·144 ±0·012		0·210 ±0·018		0·127 ±0·010	0·254 ±0·017
	Ultrasonicated	(38·9)** 0·200 *±0·024*	<0·001	(52·4) 0·321 *±0·033*	<0·001	0·127 ±0·022	0·261 ±0·030
Pectoralis major	Normal	0·271 ±0·020		0·301 ±0·017		0·170 ±0·016	0·250 ±0·017
	Ultrasonicated	(66·5) 0·454 ±0·031	<0·001	(75·0) 0·526 ±0·018	<0·001	0·174 ±0·017	0·260 ±0·017
Gastrocnemius	Normal	0·205 ±0·011		0·248 ±0·016		0·160 ±0·013	0·238 ±0·021
	Ultrasonicated	(62·9) 0·334 ±0·021	<0·001	(68·5) 0·418 ±0·026	<0·001	0·164 ±0·018	0·249 ±0·028

Values expressed as: means ± S.E. (Standard error).
* Probability of significant difference in experimental values with respect to the controls.
** Figures in parentheses represent the % increase as compared with the controls.

The data of the above two tables show that in pigeon and rat "slow" muscles, the pattern of change (increase in myofibrillar–ATPase activity) due to ultrasonication is by and large similar. The tables also show that the pattern of change due to ultrasonication in "fast" muscles is almost similar in both pigeon and rat, but the increase in myofibrillar–ATPase activity due to this treatment is much more in "fast" muscles than in "slow" muscles. There is almost no change in mitochondrial-ATPase activity after ultrasonication. It clearly indicates that mitochondrial membrane system does not act as a barrier for the substrate entrance as far as the enzyme ATPase is concerned, whereas the myofibrillar–ATPase activity increases when myofibrils are treated with ultrasonic irradiation. The results also indicate that the normal myofibrillar–ATPase activity in "fast" muscles in both pigeon and rat in general is more than the "slow" muscles, however, in pigeon there is a marked difference in the enzyme activity between a "fast" and a "slow" type of muscle.

The ATPase activity of the myosin has been correlated with the speed of muscle shortening[6], and this has been studied extensively in the muscles of different vertebrates and invertebrates. Both Ca^{++} and Mg^{++}-ATPase assays were carried out in this experimental study since it is known that Ca^{++} has a role to play in contractility of muscles whereas Mg^{++} has great physiological significance in various metabolic and regulatory processes involved in the muscle study[7].

The increase in activity of myofibrils after ultrasonication can be explained on the basis that sonic radiation is used to break the cell membranes and to release the cell contents, thus fragmentation may take place due to which the enzyme activity increases[8]. This however, may not apply to the sonicated mito-

TABLE II

Differential effects of ultrasonication on the myofibrillar and mitochondrial-ATPase activities of different skeletal muscles of rat

ATPase activity expressed as μ moles of Pi liberated/mg protein/min.

Muscle	Myofibril/ mitochondria	Specific-ATPase activity					
		Myofibrillar ATPase				Mitochondrial ATPase	
		Ca^{++}- activated	P*	Mg^{++}- activated	P*	Ca^{++}- activated	Mg^{++}- activated
Heart	Normal	0·141 ±0·018		0·152 ±0·017		0·161 ±0·015	0·240 ±0·013
	Ultrasonicated	(35)** 0·190 ±0·011	<0·001	(46) 0·250 ±0·012	< 0·001	0·168 ±0·017	0·245 ±0·012
Pectoralis major	Normal	0·158 ±0·008		0·210 ±0·010		0·201 ±0·011	0·271 ±0·107
	Ultrasonicated	(70) 0·273 ±0·016	<0·001	(47) 0·306 ±0·012	< 0·001	0·209 ±0·016	0·280 ±0·012
Soleus	Normal	0·168 ±0·018		0·226 ±0·026		0·152 ±0·010	0·218 ±0·012
	Ultrasonicated	(46) 0·252 ±0·012	<0·001	(63) 0·371 ±0·017	< 0 001	0·164 ±0·018	0·220 ±0·014

Values expressed as: means ± S.E. (Standard error).

* Probability of significant difference in experimental values with respect to the controls.

** Figures in parentheses represent the % increase as compared with the controls.

chondrial system with reference to this particular enzyme (ATPase).

The results obtained in the present study suggest a possible pattern of the differential effects of ultrasonication on the myofibrillar and mitochondrial-ATPase activity of the slow and fast muscles in birds and mammals in general. The above work is mainly confined to the study of "fast" and "slow" muscles only. Since rat is a nocturnal and pigeon is a diurnal animal, the differences in their ATPase activities of different muscles may primarily be attributed to their functional adaptation to different habits and habitats. This aspect has been reported in detail elsewhere[9].

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