TIVITY OF SARCOPLASMIC RETICULUM OF FAST AND SLOW RABBIT SKELETAL MUSCLES

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The sarcoplasmic reticulum vesicles were isolated from the three metabolic types of muscles from rabbit. The yield of coplasmic reticulum from both types of fast-twitch muscles (the psoas major and the gastrocnemius medialis) was almost three times as gh as from slow twitch red muscles (the semi membranosus proprius). Polyacrylamide gel electrophoresis profiles of sarcoplasmic iculum from fast and slow muscles showed a significant difference in the protein pattern. The calcium dependent ATPase activity and calcium uptake capacity of the fast twitch white muscle (the psoas major) were higher than in fast twitch red muscle (the strocnemius medialis). Such kinetic measurement could not be carried out in slow twitch red muscle because of their relatively low uent in sarcoplasmic reticulum.

TRODUCTION

Significant difference has been demonstrated to exist between the sarcoplasmic reticulum (SR) of fast and slow twitch mammalian eletal muscle. Morphometric studies revealed a two to three fold higher volume fraction of sarcoplasmic reticulum in fast than in slow itch fibers (TOMANEK, 1976). in accordance with this, biochemical studies demonstrated that yield of vesicular protein state and pacity of Ca^{2+} transport, Ca^{2+} dependent ATPase activity are considerably lower in sarcoplasmic reticulum from slow twitch muscle EILMANN et al., 1981 ; ABDUL WAHAB, 1988). The present study was undertaken to examine some properties of rabbit fast uscles SR and compared with the beef slow muscles SR, in view of the importance of the sarcoplasmic reticulum (SR) and Ca^{2+} in the nsformation of muscle to meat.

ATERIAL AND METHODS

New Zealand breed rabbits were used in this study, sampling of muscles began immediately after death from the psoas major as st-twitch white muscle, the gastrocnemius medialis as fast-twitch red muscle, and the semi membranosus proprius as slow twitch uscle. Muscles studied were chosen according to available information on metabolic characteristics (ABDUL WAHAB, 1984). The coplasmic reticulum (SR) vesicles from the three muscles were prepared according to the method described by LEE et al. (1979). owever, modification in the process of homogenization was made in this study. The medium contained 20 mM Hepes, pH 4, 15 mM (O, 5 mM EGTA, 5 mM $MgCl_2$ and 1% bovine serum albumin.

Protein content was determined according to LOWRY et al. (1951). Using bovine serum albumin as standard. The Ca^{2+} pendent ATPase activity was determined according to the technique described by HEILMANN et al. (1977), modified ATPase actvity as measured in the presence of 5 mM sodium azide as inhibitors of mitochondrial ATPase. The Ca^{2+} uptake of SR and the Ca^{2+} cumulation capacity of SR were determined by the method of MARTONOSI and FEROTOS (1964) modified by KIM et al. (1981). the action was started by addition of the SR suspension and stopped by filtration reaction medium on 0.22 mm pore size Millipore filter at ected time intervals.

Radioactivity of the filters was measured in a scintillation counter Beckman LS 9300. ^{45}Ca utilized from Radio Chemical Center ersham England, specific activity 10-40 mCi/mg calcium. SR proteins were analysed by SDS polyacrylamide gel electrophoresis EMMLI (1970). The same method was used to examine the purity of SR preparation by comparing the mitochondrial proteins with SR oteins.

ESULTS AND DISCUSSION

The method utilised in this study for isolating the SR faclitated a good quantity of SR (Table 1). No contamination was observed th mitochondrial proteins (Figure 1).

TABLE 1
Yield of SR from rabbit muscles

	PS	GM	SM
SR Yield	0,301	0,238	0,094
	± 0,070	± 0,031	± 0,020

Results expressed as mean ± standard error. Yield was expressed as mg protein per g fresh muscle.

The yield of SR from both types of fast-twitch muscles was almost three folds that from slow twitch red muscles HEILMANN et al., 1981 ; ABDUL WAHAB, 1989). Polyacrylamide gel electrophoresis profiles (Figure 2) of SR from fast and slow muscles showed a significant difference in the protein pattern between the fast and slow muscle vesicles concern not only the presence of additional proteins but also relative amounts.

Figure 2 (A) presents the profile of the SR from semi membranosus proprius (SM) as slow red muscle. Five major proteins bands were distinguishable (80, 57, 55, 47, 19 kd) and sixteen minors bands in particular, the bands (100, 63, 44, 12 kd). (B) profile presents the SR from gastrocnemius medialis (GM) and (C) profile presents the SR from semi membranosus proprius (SM). Three major bands were distinguishable, the first one was more than 100 kd, the other were 100 and 63 kd. As well as 15 minor bands (57, 55, 47, 44, 25 and 12 kd). The major band of 100 kd represent the Ca^{2+} dependent ATPase of SR (SARZALA et al., 1981) while the 63 kd band probably represent the calsequestrin (ZUBRZYCKA-CAARN et al., 1982). The minor band of 55 kd represent probably the polypeptide namely M55 observed by MICHALAK et al. (1980) and IKEMOTO (1982).

The Ca^{2+} dependent ATPase activity (Table 2) of fast-twitch white muscles PS were higher than of fast-twitch red muscles GM. The present results agree with those of NEWBOLD and TUME (1981) and ABDUL WAHAB (1989).

Table 3 represents Ca^{2+} accumulation and Ca^{2+} uptake of PS and GM muscle. The initial rate of Ca^{2+} uptake of SR from fast white muscle (PS) measured in the presence or absence of oxalate was always higher than that found under similar conditions in fast red muscle (GM). The total Ca^{2+} accumulation capacity was also higher in the former muscles than in GM muscles, in accordance with KIM et al. (1981) and NEWBOLD and TUME (1981). The PS represent an accumulation capacity equal to 23,325 mmole Ca^{2+}/mg protein in 1 mM Ca^{2+} concentration but the GM represent only 15,75 mmole Ca^{2+}/mg protein. This accumulation is 11,358 and 6,98 mmole Ca^{2+}/mg protein respectively in 0,4 mM Ca^{2+} concentration.

TABLE 2

Ca^{2+} dependent ATPase activities of SR from rabbit muscles.

	PS	GM
Ca^{2+} dependent ATPase	1,749 ± 0,48	0,416 ± 0,070

ATPase activities are expressed in µmoles Pi/mn/mg protein
- PS Psoas major - GM Gastrocnemius medialis

TABLE 3

$^{45}Ca^{2+}$ accumulation of SR from rabbit muscles

Ca^{2+} uptake kinetics	PS	GM
Initial velocity - 10 mM oxalate	9,708 ± 0,404	8,00 ± 0,54
Initial velocity without oxalate	0,900 ± 0,008	0,616 ± 0,128
Initial velocity - 10 mM oxalate + EGTA	4,56 ± 0,32	3,864 ± 0,512
$^{45}Ca^{2+}$ accumulation - ^{45}Ca (1 mM)	23,325 ± 2,90	15,75 ± 1,35
capacity - ^{45}Ca (0,4 mM)	11,358 ± 0,371	6,98 ± 1,67

Initial velocity was expressed as µmole Ca^{2+}/mg protein/min $^{45}Ca^{2+}$ accumulation capacity was expressed as µmole Ca^{2+}/mg protein. The free calcium concentration was calculated using an apparent binding constant of 1 mM EGTA.

CONCLUSION

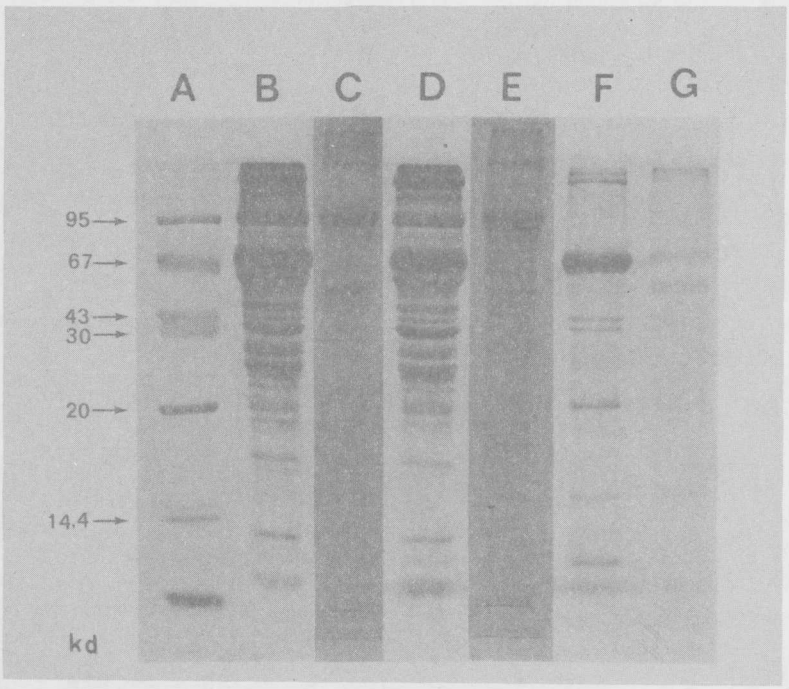
The results of this experiment have shown that the yield of sarcoplasmic fast twitch muscles was almost three times as high as those from slow twitch red muscles. Polyacrylamide gel electrophoresis profiles of sarcoplasmic reticulum from fast and slow muscles showed a significant difference in the protein pattern. The Ca^{2+} dependent ATPase activity, Ca^{2+} uptake and Ca^{2+} accumulation capacity of the fast twitch white muscles (PS) were higher than in the fast twitch red muscle (GM).

Such kinetic measurements could not be carried out in the slow twitch red muscles (SM) because of their relatively low content of sarcoplasmic reticulum.

REFERENCES

ABDUL WAHAB E.J., 1984. "Thèse de Docteur-Ingénieur". Université de Clermont-Ferrand II.

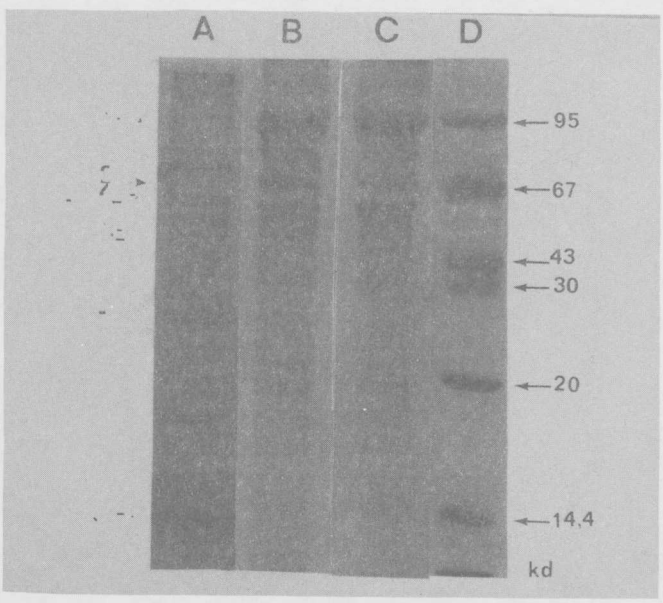
ABDUL WAHAB E.J., 1989. "Activity of sarcoplasmic reticulum of fast and slow rabbit skeletal muscles". 35th International Congress of Meat Science and Technology, proceeding 3, 1099-1104.



ure 1 : Polyacrylamide gel electrophoretic profiles of SR and mitochondrial proteins of rabbit muscles.

A - Standard proteins : phosphorylase b (95 Kd), BSA (67 Kd), ovalbumine (43 Kd), carbonic anhydrase (30 Kd), soja trypsine ibitor (20 Kd), α lactalbumin (14,4 Kd).

B - Psoas major mitochondria
C - Psoas major SR
D - Gastrocnemius medialis mitochondria
E - Gastrocnemius medialis SR
F - Semi membranosus proprius mitochondria
G - Semi membranosus proprius SR



ure 2 : Polyacrylamide gel electrophoretic profiles of SR from rabbit muscles.

A - Semi membranosus proprius
B - Gastrocnemius medialis
C - Psoas major
D - Standard proteins : as same as in Fig. 1-

1 - Ca^{2+} dependent ATPase
2 - Calsequestrin
3 - M55 - protein
4 - 57 Kd
5 - 47 Kd
6 - 44 Kd
7 - 25 Kd
8 - 12 Kd

FISK C.H., SUBBAROW Y., 1925. "The colorimetric determination of phosphorus". J. Biol. Chem., 66 375-382.

HEILMANN C., PETTE D., 1979. "Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation". Eur. J. Biochem., 93, 437-441.

HEILMANN C., MULLER W., PETTE D., 1981. "Correlation between ultrastructural and functional changes in sarcoplasmic reticulum during chronic stimulation of fast muscle". J. Membrane Biol., 59, 143-149.

IKEMOTO., 1982. "Structure and function of the calcium pump protein of sarcoplasmic reticulum. Ann. Rev. Physiol., 44, 297-317.

KIM D.H., WITZMANN F.A., FITTS R.H., 1981. "A comparison of sarcoplasmic reticulum function in fast and slow skeletal muscle using crude homogenate and isolated vesicles". Life Sci., 28, 2223-2229.

KIM D.H., WIBLE G.S., WITZMANN F.A., FITTS R.H., 1981. "The effect of exercise training on sarcoplasmic reticulum function in fast and slow skeletal muscle. Life Sci., 28, 2671-2676.

LOWRY O.Y., ROSENBROUGH J.J., FARR A.L., RANDALL R.J., 1951. "Protein measurement with the folin phenol reagents". J. Biol. Chem., 193, 265-275.

LAEMMLI U.K., 1970. "Cleavage of structural proteins during assembly of the head of bacteriophage T4". Nature, 227, 680-685.

LEE C.P., MARTENS M.E., JANKULOVSKA L., NEYMARK M.A., 1979. "The uptake of Ca^{2+} by sarcoplasmic reticulum fragments muscle and nerve". 2, 340-348.

MARTONOSI A., FEROTOS R., 1964. "Sarcoplasmic reticulum, 1. The uptake of Ca^{2+} by sarcoplasmic reticulum fragments". J. Biol. Chem., 239, 648-658.

MARTONOSI A.N., CHYN T.L., SCHIBECI A., 1978. "The calcium transport of sarcoplasmic reticulum". Annals. New-York Academy of Sciences, 148-157.

MICHALAK M., CAMPBELL K.P., MACLENNAN D.H., 1980. "Localization of the high affinity calcium binding protein and intrinsic glycoprotein in sarcoplasmic reticulum membranes". J. Biol. Chem., 255, 1317-1326.

NEWBOLD R.P., TUME R.K., 1981. "Comparison of the effects of added orthophosphate on calcium uptake and release by bovine and rabbit muscle sarcoplasmic reticulum". J. Food. Sci., 46, 5, 1327-1332.

TOMANEK R.J., 1976. "Ultrastrucrure differentiation of skeletal muscle fibers and their diversity". J. Ultrastruct. Res., 55, 212-217.

ZNBRZYCKA-GAARN E., KORCZAK B., OSINSKA H., SATZALA M.G., 1982. "Studies on sarcoplasmic reticulum from slow twitch muscle". J. Muscle research and cell motolity, 3, 191-212.