Calcium Accumulating Ability of Mitochondria from Bovine Coronary Artery Comparison with Aortic Mitochondria

Satoshi TAKEO, Ph.D. *and* Matao SAKANASHI, M.D.

SUMMARY

The calcium accumulating ability of mitochondria isolated both from bovine coronary artery and aorta was investigated. Coronary artery and aorta were pretreated with 0.1% collagenase. Cytochrome c oxidase activities of mitochondria isolated from coronary artery and aorta showed 25-fold and 19-fold increases, respectively, as compared with those of each homogenate, whereas NADPH-cytochrome c reductase, potassium-phosphatase and Na⁺-K⁺ ATPase activities increased less than 2-fold. This suggests that the isolation procedure is capable of obtaining a subcellular fraction highly enriched with mitochondria.

Mitochondrial calcium uptake activity of the coronary artery was approximately 250 nmoles Ca²⁺/mg protein/10 min, and was markedly depressed with metabolic inhibitors such as NaN₃, ruthenium red and 2,4-dinitrophenol. Calcium uptake activity of bovine aortic mitochondria showed similar activity and a similar trend in sensitivity to metabolic inhibitors. By contrast, the onset of the calcium binding reaction of the aortic mitochondria was slower and the azide-sensitivity of the mitochondria to magnesium ATPase activity was lower than those for coronary artery mitochondria. The present study has provided a method for isolation of mitochondria with a high capacity of calcium uptake activity, which may prove meaningful for future physiological and pharmacological evaluation of mitochondrial calcium accumulation in vascular smooth muscle.

Additional Indexing Words:

Aorta Calcium binding Calcium uptake Coronary artery
Mitochondria

From the Department of Pharmacology, School of Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa 903-01, Japan.

Address for reprint: Matao Sakanashi, M.D., Department of Pharmacology, Faculty of Medicine, University of the Ryukyus, Okinawa 903-01, Japan.

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IT is well established that calcium ions play a critical role in regulating both striated and smooth muscle contraction. In striated muscle, the sarcoplasmic reticulum is well developed and considered to be an important source for intracellular free calcium during excitation-contraction coupling.¹⁾ However, the sarcoplasmic reticulum of some vascular smooth muscle is poorly developed,²⁾⁻⁴⁾ suggesting that some other subcellular organelles, such as the mitochondria and the cell membrane, may be important sources for intracellular calcium. There are several reports regarding calcium accumulation by mitochondria from rat and human myometrium,^{5),6)} bovine pulmonary artery and mesenteric artery,⁷⁾ dog trachea⁸⁾ and rabbit aorta.⁹⁾ However, these reports provided less data concerning physiological and pharmacological characteristics of calcium accumulating ability of vascular smooth muscle than of cardiac muscle. Furthermore, characterization of mitochondria from the coronary artery has not been extensively demonstrated in the literature.^{9),10)} The present study was undertaken to explore the calcium accumulating ability of mitochondria from bovine coronary artery. For the purpose of comparison, the calcium accumulating ability of bovine aortic mitochondria, isolated under the same conditions, was also examined.

METHODS

Isolation of mitochondria

Bovine heart and aorta were obtained from a slaughter house. It took 5-15 min to remove these tissues after exsanguination. The heart and aorta were put into cold saline and transferred to the laboratory. Fat and connective tissues were trimmed away from the aorta. About one third of the aortic adventitia was removed to eliminate the fat-enriched portion of the tissue. By contrast, the coronary artery was carefully isolated by supporting several branches of coronary artery such as the left circumflex, left anterior descending and right coronary arteries, with acrylic bars before trimming off the myocardium. After weighing the wet weight (6-8 Gm of each tissue), the arteries were finely minced with scissors. The minced tissues were suspended in 3 volumes (estimated from the wet tissue weight) of a medium containing 120 mM NaCl, 5 mM MgCl₂, 1.25 mM CaCl₂ and 20 mM imidazole (pH 7.4). The suspension was preincubated at 37°C for 5 min, and collagenase was added into the suspension to obtain a 0.1% collagenase solution. The suspension was incubated for 10 min at 37°C, then chilled in an ice-cold bath (0-4°C). The suspension was immediately centrifuged at 1,500×g for 2 min, and supernatant was discarded. The resulting minced tissue was washed once with 0.25 M sucrose+10 mM imidazole (pH 7.4) and homogenized for

5 sec by an Ultraturrax (Werke AG) at one third maximal speed. The homogenate was filtered through 2 layers of gauze, then the residual tissue was rehomogenized under the same conditions. The homogenate was combined with the previous filtrate (Fraction 1) and centrifuged at $1,500\times g$ for 15 min. The supernatant was filtered again through 4 layers of gauze to eliminate fat and then centrifuged at $12,000\times g$ for 20 min. The sediment (Fraction 2) was gently suspended in 0.25 M sucrose — 1 mM EDTA — 0.5% albumin (pH 7.0) and centrifuged at $10,000\times g$ for 10 min. The resulting pellet (Fraction 3) or Fraction 2 was finally suspended in 0.25 M sucrose — 10 mM imidazole (pH 7.0) at a concentration of 1.0–1.5 mg/ml, and employed for assay of mitochondrial biochemical activity. The isolation procedure was carried out at 0–4°C. Further washing of Fraction 3 with a medium containing either 0.25 M sucrose — 1 mM EDTA — 0.5% albumin, or 0.25 M sucrose — 10 mM imidazole did not produce an appreciable increase in the biochemical activity of mitochondria. Treatment of Fraction 2 with a medium containing 0.18 M KCl — 10 mM EDTA — 0.5% albumin (pH 7.0), which has been commonly employed for isolation of heart mitochondria,¹²⁾ attenuated the calcium accumulating activity for mitochondria of the vascular smooth muscle.

Measurements of marker enzyme activity

Measurements of marker enzyme activity were performed according to methods described elsewhere.^{13)–18)} For this purpose, each fraction was well homogenized again to explore latent activities. Cytochrome c oxidase activity was estimated by the method of Wharton and Tzagoloff,¹³⁾ and the activity was expressed as the estimated first-order velocity constant (nmoles cytochrome/min/mg protein). NADPH-cytochrome c reductase activity (nmoles NADPH/min/mg protein) was determined by the method of Masters et al.¹⁴⁾ The measurement of potassium-stimulated phosphatase (nmoles nitrophenol/min/mg protein) was performed by the method of Lamers et al.¹⁵⁾ 5'-Nucleotidase¹⁶⁾ and ouabain-sensitive $\text{Na}^+\text{-K}^+$ ATPase¹⁷⁾ activities were estimated by determining inorganic phosphate liberated during incubation according to the method of Taussky and Shorr.¹⁸⁾ The activities are shown as nmoles Pi/min/mg protein. Protein concentrations were determined by the method of Lowry et al.¹⁹⁾

Measurements of ATPase activity

The ATPase assay was performed according to the method described elsewhere.²⁰⁾ Magnesium-dependent ATPase (Mg-ATPase) activity was measured in a medium containing 100 mM KCl, 20 mM Tris-HCl, 10 mM MgCl_2 , 0.2 mM EGTA, 4 mM Tris-ATP and about 0.1 mg protein (pH 6.8). After 3 min-preincubation, the reaction was initiated by adding 4 mM ATP

and, after 5 min of incubation, terminated by adding 12% trichloroacetic acid. The resulting mixture was centrifuged at 4°C at 1,000×g for 10 min and the supernatant was sampled for estimation of inorganic phosphate according to the method of Taussky and Shorr.¹⁸⁾ Total ATPase activity was measured by the same procedure as Mg-ATPase activity except that 0.1 mM CaCl₂ was substituted for 0.2 mM EGTA. The difference in the activities between the total ATPase and the Mg-ATPase was taken as calcium-stimulated, magnesium-dependent ATPase (Ca-ATPase) activity. Mg-ATPase activity was also measured in the presence of 5 mM NaN₃ in order to determine the azide-sensitivity of Mg-ATPase.

Calcium accumulating activity

The measurement of calcium accumulating activity was performed by a method described elsewhere.²⁰⁾ Calcium binding activity was measured at 25°C for 10 min in a medium containing 10 mM KCl, 20 mM Tris-HCl, 10 mM MgCl₂, 4 mM Na₂-ATP, 0.1 mM ⁴⁵CaCl₂ and about 0.15 mg protein (pH 6.8), unless otherwise mentioned. The measurement of calcium uptake activity was conducted at 37°C for 10 min in a medium containing 10 mM KCl, 20 mM Tris-HCl, 10 mM MgCl₂, 4 mM Na₂-ATP, 0.1 mM ⁴⁵CaCl₂, about 0.15 mg protein and permeant ion(s) such as either 4 mM potassium oxalate or 4 mM succinate + 4 mM KH₂PO₄ (pH 6.8). The reaction was initiated by adding ⁴⁵CaCl₂ and terminated by Millipore filtration methods described elsewhere.²⁰⁾ Calcium uptake activity was also estimated in the presence of metabolic inhibitors such as NaN₃, ruthenium red and dinitrophenol. Both calcium binding and uptake activities were measured in the same manner as above, under different concentrations of ATP (0.3 to 4 mM) and CaCl₂ (5 to 100 μM), incubation times (2 to 30 min) and pH (6.5 to 7.8). Less than 10% of ATP added to the medium was consumed in the assay.

Statistical methods and reagents employed

The Student's t-test was used for comparison of the values for coronary artery with those for aorta. Differences at the 95% confidence level were considered significant. Reagents employed in the present experiment were collagenase (Type I: Sigma Co.), protease (Nagarse, Nagase Sangyo), ruthenium red (Sigma Co.), 2, 4-dinitrophenol (Sigma Co.) and cytochrome c (Type III: Sigma Co.). ⁴⁵CaCl₂ was purchased from the Japan Radioisotope Reagent Association.

RESULTS

Preliminary study

Cytochrome c oxidase activity was used as a marker for the mitochon-

drial fraction in the present experiments. First, two commercially available enzymes, collagenase and protease (Nagarse) were employed to soften the vascular smooth muscle, since tissue rigidity is one of the most difficult problems for isolation of subcellular fractions. The coronary artery was treated for 10 min at 37°C either with 0.1% collagenase or 0.1% protease, or without any enzymes (non-treatment). The cytochrome c oxidase activities of Fraction 3 from the coronary artery treated with collagenase, protease and without enzymes were 622, 580 and 501 nmoles/min/mg protein, respectively (mean values of 2 experiments). When the tissue was incubated for longer than 15 min or shorter than 10 min at 37°C, the cytochrome c oxidase activity of Fraction 3 was found to be lower than that at a 10 min-incubation. A similar trend in the cytochrome c oxidase activity was found for Fraction 3 from the bovine aorta. Thus, we employed a 10 min-incubation with 0.1% collagenase at 37°C for the pretreatment of the vascular smooth muscle.

Marker enzyme study

In the first set of experiments, a marker enzyme study was performed using different fractions obtained in the isolation procedure for mitochondria (Table I). NADPH-cytochrome c reductase activity was employed as a marker for the microsomal fraction.¹¹⁾ 5'-Nucleotidase activity is found in the microsomal fraction and/or sarcolemmal fraction,²¹⁾ whereas potassium phosphatase and ouabain-sensitive Na⁺-K⁺ ATPase activities are considered to be specific for the sarcolemmal fraction. Control values for cytochrome c oxidase

Table I. Marker Enzyme Activities and Protein Yield of Fractions Obtained from the Isolation Procedure for Mitochondria

	Fraction 1		Fraction 2		Fraction 3	
	Coronary artery	Aorta	Coronary artery	Aorta	Coronary artery	Aorta
Cytochrome c oxidase	24 ± 3	25 ± 3	550 ± 64	470 ± 46	595 ± 50	490 ± 52
NADPH-cytochrome c reductase	136 ± 12	186 ± 15	238 ± 19	244 ± 18	172 ± 15	213 ± 16
5'-Nucleotidase	33 ± 1	32 ± 4	52 ± 11	78 ± 11	27 ± 3	34 ± 3
K ⁺ -stimulated phosphatase	5 ± 2	5 ± 2	9 ± 2	10 ± 1	6 ± 1	7 ± 2
Na ⁺ -K ⁺ ATPase	18 ± 4	19 ± 2	12 ± 1	15 ± 1	17 ± 5	22 ± 4
Protein yield			0.092 ± 0.010	0.101 ± 0.010	0.084 ± 0.009	0.091 ± 0.009

Units of the activities were as follows: Cytochrome c oxidase (nmoles cytochrome/min/mg protein), NADPH-cytochrome c reductase (nmoles NADPH/min/mg protein), 5'-nucleotidase (nmoles Pi/min/mg protein), K⁺-stimulated phosphatase (nmoles nitrophenol/min/mg protein), Na⁺-K⁺ ATPase (nmoles Pi/min/mg protein). Each value of the enzyme activities represents a mean ± S.E.M. of 4-6 experiments. Protein yield was expressed as a percentage mg protein yield, to the initial tissue wet weight (n=12).

activity of Fraction 3 (mitochondrial fraction) from bovine coronary artery were approximately one sixth of the values for bovine heart mitochondria ($3.62 \mu\text{moles/min/mg}$ protein, a mean value of 2 experiments). The cytochrome c oxidase activity of Fraction 3 from coronary artery was approximately 25 times higher than the value for the homogenate, whereas that of aorta was 19 times. By contrast, increases in NADPH-cytochrome c reductase activity of Fraction 3 of both coronary artery and aorta increased to less than 2 times, compared with values for the homogenate. The NADPH-cytochrome c reductase activity, measured in the presence of $1 \mu\text{M}$ rotenone, was slightly depressed (7%). Furthermore, 5'-nucleotidase and potassium-stimulated phosphatase activities of Fraction 3 in both tissues were almost the same as in the homogenates. $\text{Na}^+\text{-K}^+$ ATPase activities of Fraction 3 of coronary artery and aorta were also similar to those for the homogenate. Treatment of the crude mitochondrial fraction (Fraction 2) with a buffer containing sucrose, EDTA and albumin elicited an increase in cytochrome c oxidase activity, whereas NADPH-cytochrome c reductase and 5'-nucleotidase activities were decreased by the treatment. The protein yield of Fraction 3 from bovine coronary artery was almost identical to that from the coronary artery, and extremely low as compared with the protein yield for rat heart muscle mitochondria ($0.22 \pm 0.01\%$, $n=10$).²⁰⁾

ATPase activity

As shown in Table II, the mitochondrial Mg-ATPase activity of the aorta was significantly higher than that of the coronary artery (30% higher). However, the mitochondrial Ca-ATPase activity of the aorta was essentially identical to that of the coronary artery. The Mg-ATPase activity of the aorta, measured in the presence of 5 mM NaN_3 , was almost identical to that of the coronary artery. However, the ratio of azide-sensitivity to Mg-ATPase activity of coronary artery mitochondria was 83–100%; the value for the aorta

Table II. ATPase Activities of the Mitochondrial Fraction (Fraction 3) from the Bovine Coronary Artery and Aorta

	Coronary artery	Aorta
Mg-ATPase	153 ± 13	$199 \pm 16^*$
Ca-ATPase	20 ± 6	24 ± 6
Azide-sensitive Mg-ATPase	144 ± 3 ($93 \pm 2\%$)	131 ± 32 ($66 \pm 2\%$)*

The Mg-ATPase and Ca-ATPase activities were measured in the presence of 10 mM MgCl_2 , and 10 mM MgCl_2 – 0.1 mM CaCl_2 , respectively. The activities were expressed as $\text{nmoles Pi/min/mg protein}$.

* Significantly different from the coronary groups. Each value represents a mean $\pm$ S.E.M. of 6 experiments.

Fig. 1. Calcium binding activity of the mitochondrial fraction isolated from coronary artery (●) and aorta (Δ) at different incubation times (nmoles Ca^{2+} /mg protein). The incubating conditions are described in the methods section. Each value represents a mean $\pm$ S.E.M. of 5 experiments.

was 55–70%. The Mg-ATPase activities of Fraction 2 from coronary artery and aorta were 15–21% lower than the values from Fraction 3.

Calcium binding activity

Mitochondrial calcium binding activities of both coronary artery and aorta at times of incubation ranging from 0 to 30 min are shown in Fig. 1. The calcium binding activity of coronary artery mitochondria showed a rapid rise after the onset of incubation and reached the maximal level after a 20 min-incubation. The calcium binding activity of aortic mitochondria increased in an almost linear manner for incubation times up to 30 min, but it was significantly lower during 20 min of incubation than the coronary artery mitochondria. Since the calcium binding of aortic mitochondria at 45 min-incubation was approximately 170 nmoles Ca^{2+} /mg protein, the activity appears to be saturable. In the absence of ATP in the incubation medium, the calcium binding activities of coronary artery and aortic mitochondria were 28 ± 4 and 16 ± 2 nmoles Ca^{2+} /mg protein/10 min, respectively ($n=4$).

Calcium uptake activity

a) Effects of permeant ions and incubation time

The calcium uptake activities of coronary artery and aortic mitochondria were measured at different times of incubation in the presence of either 4 mM oxalate or 4 mM succinate+4 mM KH_2PO_4 (Fig. 2). In the presence of

Fig. 2. Calcium uptake activity of the mitochondrial fraction isolated from the coronary artery (○) and aorta (△) at different times of incubation in the presence of 4 mM succinate + 4 mM KH_2PO_4 , and from the coronary artery (●) and aorta (▲) in the presence of 4 mM potassium oxalate (nmoles Ca^{2+} /mg protein). Each value represents a mean $\pm$ S.E.M. of 5 experiments.

oxalate, the calcium uptake activity of coronary artery mitochondria was slightly higher than that of aortic mitochondria. In the presence of succinate + phosphate, the calcium uptake activities of both coronary artery and aortic mitochondria were essentially identical except for the values at 30 min-incubation, where the activity of aorta was lower than that of coronary artery fraction. Calcium uptake by mitochondria from both coronary artery and aorta in the presence of succinate + phosphate was of rapid onset and high capacity in comparison with that in the presence of oxalate. The calcium uptake activity of coronary artery and aortic mitochondria was found to be saturable after 20 or 30 min of incubation. The calcium uptake activity of Fraction 2 was 70–93% of the Fraction 3 value.

b) Effects of CaCl_2 concentration

The calcium uptake activity of mitochondria isolated from bovine coronary artery and aorta was also measured in the medium containing various concentrations of CaCl_2 (Table III). There were no significant differences in the calcium uptake activity between coronary artery and aortic mitochondria at 5 to 100 μM of CaCl_2 .

Table III. Calcium Uptake Activities at Different Concentrations of CaCl_2 of the Mitochondrial Fraction (Fraction 3) Isolated from the Bovine Coronary Artery and Aorta

Ca^{2+} (μM)	Coronary artery	Aorta
5	11.3 ± 1.4	11.4 ± 1.0
10	23.4 ± 2.2	23.5 ± 1.3
30	87.6 ± 6.8	71.7 ± 6.5
50	147.4 ± 8.0	128.7 ± 7.7
100	285.2 ± 22.0	263.0 ± 12.3

The activities were measured for 10 min in the presence of 4 mM ATP, 10 mM MgCl_2 and different concentrations of CaCl_2 , and expressed as nmoles Ca^{2+} /mg protein/10 min.

Each value represents a mean $\pm$ S.E.M. of 4-5 experiments.

Table IV. Calcium Uptake Activities in the Presence of Metabolic Inhibitors of the Mitochondrial Fraction (Fraction 3) Isolated from the Bovine Coronary Artery and Aorta

	Control	-ATP	NaN_3	Dinitrophenol	Ruthenium red
Coronary artery	254 ± 16	38 ± 8	23 ± 5	79 ± 10	5 ± 2
Aorta	266 ± 20	32 ± 13	14 ± 4	80 ± 8	21 ± 5

Mitochondrial calcium uptake activity was measured in the standard medium (methods section) in the presence of either 5 mM NaN_3 , 0.1 mM dinitrophenol or 1 μM ruthenium red, and in the absence of inhibitors (control) and ATP (-ATP). The activities were expressed as nmoles Ca^{2+} /mg protein/10 min.

Each value represents a mean $\pm$ S.E.M. of 4-5 experiments.

c) Effects of inhibitors

The effects of inhibitors on mitochondrial calcium uptake activity were examined in the presence of 100 μM CaCl_2 and succinate+phosphate (Table IV). The ATP-independent calcium uptake activities of coronary artery and aortic mitochondria were 15 and 12% of the ATP-dependent calcium uptake activity (control value). Incubation with 5 mM NaN_3 showed a more than 90% inhibition in mitochondrial calcium uptake activity, while 0.1 mM dinitrophenol depressed the calcium uptake activities of coronary artery and aortic mitochondria by approximately 70%. The calcium uptake activities of coronary artery and aortic mitochondria were almost completely inhibited in the presence of 1 μM ruthenium red. Furthermore, 0.1 μM ruthenium red inhibited the calcium uptake activity of coronary artery and aortic mitochondria by 67 ± 4 and $72 \pm 5\%$, respectively ($n=5$).

d) Effects of ATP concentration

Mitochondrial calcium uptake activity was measured in the presence of different concentrations of ATP (0.3 to 4 mM) (Fig. 3). Lineweaver-Burk

plots of the results indicate that K_m values for the calcium uptake activity of the coronary artery and aortic mitochondria were almost similar ($K_m=0.2$ mM ATP), although the V_{max} for aortic mitochondria (278 nmoles Ca^{2+} /mg protein) was slightly higher than that of coronary artery mitochondria (260 nmoles Ca^{2+} /mg protein) at an incubation time of 10 min.

Fig. 3. Calcium uptake activity of the mitochondrial fraction isolated from the coronary artery (○) and aorta (●) at different concentrations of ATP (nmoles Ca^{2+} /mg protein/10 min), and their Lineweaver-Burk plots. The reaction was carried out for 10 min in the presence of 0.1 mM CaCl_2 . The results were typical of 3 experiments.

Fig. 4. Calcium uptake activity of the mitochondrial fraction isolated from the coronary artery (○) and aorta (▲) at different pH (nmoles Ca^{2+} /mg protein/10 min). The reaction was carried out for 10 min in the presence of 0.1 mM CaCl_2 . Each value represents a mean $\pm$ S.E.M. of 4 experiments.

e) Effects of pH

Mitochondrial calcium uptake activities were measured in the incubation medium over pH values ranging from 6.5 to 7.8. As shown in Fig. 4, the calcium uptake activities of mitochondria from the coronary artery increased with a rise in pH of the incubation medium, but the uptake of aortic mitochondria is almost similar up to pH 7.4. Calcium uptake activities of both coronary artery and aortic mitochondria tended to be higher at pH 7.8 than at pH 7.4.

DISCUSSION

In preliminary studies, collagenase and protease were used for the pretreatment of smooth muscle tissue. Both enzyme treatments yielded purer mitochondrial fractions than the isolation without enzyme treatment on the basis of their cytochrome c oxidase activities. The cytochrome c oxidase activity of Fraction 3 (mitochondrial fraction), isolated after pretreatment with collagenase, was slightly higher than with protease. Thus, smooth muscles were treated with 0.1% collagenase in the present studies. However, our results suggest that protease treatment may also represent a satisfactory procedure in the isolation of vascular smooth muscle mitochondria.

Since mitochondrial fractions isolated from the bovine heart by the method of Sordahl¹²⁾ show about a 4-fold increase in cytochrome c oxidase activity as compared with the homogenate, the present results concerning marked increases in cytochrome c oxidase activity (19 to 25-fold) suggest that the isolation procedure employed in the present experiment provided a highly purified mitochondrial fraction. This was also supported by our findings that the increment in NADPH-cytochrome c reductase activity was only 2-fold and that 5'-nucleotidase and potassium-stimulated phosphatase activities were similar to values from crude homogenate. When Fraction 2 was washed with 0.25% sucrose — 1 mM EDTA — 0.5% albumin, cytochrome c oxidase activity increased slightly, but NADPH-cytochrome c reductase and 5'-nucleotidase activities decreased. This indicates that the washing procedure is necessary in order to obtain a fraction highly enriched with mitochondria. The cytochrome c oxidase activity of mitochondria derived from the coronary artery was higher than that from the aorta, suggesting that the mitochondrial fraction isolated from the coronary artery may be more enriched. This is also supported by our findings of a relatively low inhibitory action of NaN_3 on the Mg-ATPase activity of Fraction 3 derived from the aorta (66%), as compared with the coronary artery fraction (93%).

Zelick et al¹⁰⁾ demonstrated that calcium uptake activity of mitochondria

isolated from the pig coronary artery was about 20 nmoles Ca^{2+} /mg protein/10 min, when measured at 25°C in the presence of 0.1 mM CaCl_2 , 5 mM ATP, 5 mM oxalate and 5 mM MgCl_2 . Fitzpatrick et al⁹⁾ reported that the net calcium uptake by mitochondrial fractions from rabbit aorta was about 25 nmoles Ca^{2+} /mg protein/10 min, when measured at 37°C in the presence of 3 mM Mg-ATP, 5 mM NH_4 -oxalate and 0.1 mM CaCl_2 . By contrast, calcium uptake activities of mitochondria from both bovine coronary artery and aorta in the present experiments were 254 and 266 nmoles Ca^{2+} /mg protein/10 min, respectively, when measured at 37°C in the presence of 4 mM ATP, 10 mM MgCl_2 , 0.1 mM CaCl_2 , 4 mM K-succinate and 4 mM KH_2PO_4 . Although differences in incubating conditions and compositions, and species differences should be taken into consideration, it is noteworthy that the mitochondrial fraction isolated from bovine coronary artery and aorta showed very high calcium uptake activities as compared with mitochondrial fractions isolated from other vascular smooth muscle tissues. Thus, the present experimental procedure may provide a meaningful method for the evaluation of calcium accumulation of vascular smooth muscle mitochondria.

Takeo et al²⁰⁾ demonstrated that mitochondrial fraction isolated from the rat heart showed a K_m of about 0.2 mM ATP for calcium uptake activity: the calcium uptake activity was independent of pH in the incubation medium. In this respect, our findings on calcium uptake activity of vascular smooth muscle mitochondria revealed a similar K_m value to rat heart mitochondria, but the dependency of mitochondrial calcium uptake on the pH of the medium was slightly different from that of the heart mitochondria.²⁰⁾

Differences between the mitochondria derived from bovine coronary artery and aorta were seen in the rate of onset of activation of calcium binding and in the ratio of azide-sensitivity to Mg-ATPase activity. Although these differences may be partly due to different purity of these mitochondrial preparations, we cannot rule out the possibility that these data reflect different properties of the membrane *per se*. However, mitochondria derived from bovine coronary artery and bovine aorta displayed similar calcium uptake activity under normal incubating conditions, in the presence of inhibitors and at different concentrations of CaCl_2 . Thus, it is unlikely that marked differences in calcium accumulating ability exist between the two mitochondrial fractions. Nonetheless, responsiveness of the mitochondria to various pharmacological and biochemical agents and the mitochondrial alterations under a variety of pathophysiological conditions should be further examined to elucidate properties of mitochondria from bovine coronary artery and aorta.

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