Developmental Regulation of the Sarcoplasmic Reticulum Calcium Pump in the Rabbit Heart

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ABSTRACT. Previous studies have demonstrated that myocardial function changes during mammalian perinatal development. The purpose of this study was to evaluate the subcellular basis underlying the slower relaxation in the developing heart by examining perinatal changes in sarcoplasmic reticulum (SR) function, and in SR Ca^{2+} pump protein and mRNA abundance. We measured Ca^{2+} uptake and ATPase rates in isolated fetal, newborn, and adult rabbit cardiac SR membranes. In fetal and adult SR membranes, we estimated the active Ca^{2+} pump protein content by measuring the steady state Ca^{2+}-dependent phosphoenzyme content; the total Ca^{2+} pump protein content was estimated by Western analysis of the immunoreactive Ca^{2+} pumps. We isolated RNA from fetal and adult hearts and estimated the SR Ca^{2+} pump mRNA content by Northern analysis. Ca^{2+} uptake and ATPase rates were significantly lower in the fetal and newborn SR membranes compared with the adult. The contents of active and total Ca^{2+} pump protein and of Ca^{2+} pump mRNA were 52–63% lower in the fetus than in the adult. These results indicate that a great deal of the slower sarcoplasmic reticulum Ca^{2+} uptake and ATPase rates in the fetal rabbit heart can be related to lower Ca^{2+} pump mRNA and protein contents. It is evident that transcriptional and/or posttranscriptional regulation of the SR Ca^{2+} pump may form an important part of the subcellular basis of the perinatal change in mammalian cardiac relaxation. (*Pediatr Res* 31: 474–479, 1992)

Abbreviations

SR, sarcoplasmic reticulum
Ca^{2+}, calcium
cRNA, complementary RNA

The maximum rate of cardiac relaxation is slower in the developing mammalian heart than in adults of the same species (1). The rate of cardiac relaxation is a function of the rate of removal of Ca^{2+} from the myofibrils, which in the adult is regulated primarily by the ATP-dependent transport of Ca^{2+} from the cytosol into the SR lumen by the SR Ca^{2+} pump (2–4). The slower cardiac relaxation in the developing heart has been associated with slower Ca^{2+} uptake and ATPase rates in isolated cardiac SR vesicles from fetal rabbits and sheep (5–8). The demonstration of a single gene for the cardiac SR Ca^{2+} pump that expresses a single protein product in the heart (9–11) raised

the possibility that the slower SR Ca^{2+} transport function in the developing heart could be the result of lower Ca^{2+} pump mRNA and protein contents. Komuro *et al.* (12) demonstrated that the SR Ca^{2+} pump mRNA content was lower in the fetal rat heart compared with the adult. However, there have been discrepancies in the published data for the Ca^{2+} pump protein content during perinatal cardiac development. Komuro *et al.* (12) found no significant difference between fetal and adult rat cardiac Ca^{2+} pump protein contents, whereas Mahoney *et al.* (5) and Pegg *et al.* (7) demonstrated a significantly lower Ca^{2+} pump protein content in fetal than in adult sheep. We have reevaluated this issue in rabbits with a well-characterized cardiac SR membrane preparation. The results suggest that a great deal of the slower SR Ca^{2+} transport function in the developing rabbit heart can be related to lower cardiac Ca^{2+} pump mRNA and protein contents.

MATERIALS AND METHODS

All of the protocols used in this study were performed according to the guidelines in the National Institutes of Health Guide for The Care and Use of Laboratory Animals, with the highest standards of humane care. They were approved by the Committee on Animal Experimentation at Baylor College of Medicine.

Isolation and characterization of SR membranes. Cardiac SR vesicles were isolated at the same time from 29-d gestation New Zealand White rabbit fetuses (term = 31 d), 1- to 2-d-old newborns, and their mothers by a modification of the method of Harigaya and Schwartz (13). The mothers were anesthetized with 75 mg/kg ketamine and xylazine, and 2.5 mg/kg of acepromazine. Fifteen to 20 min later, the mothers underwent cervical subluxation. The fetal and maternal hearts were removed rapidly, rinsed in an iced solution of 0.9% saline, and trimmed free of atria, right ventricular free wall, and epicardial fat. The remaining left ventricular free wall and septum was washed three times in a solution of 10 mM $NaHCO_3$ and 10 mM Tris maleate (pH 6.8, 4°C), minced, and homogenized in 10 vol of the same solution [15 s × 3 at 5 800 rpm with a PT3000 Brinkman Polytron (Westbury, NY)]. The homogenate was centrifuged for 5 min at 650 × g, and 15 min at 4 000 × g. The supernatant was centrifuged for 20 min at 8 000 × g, and the resulting supernatant was centrifuged at 40 000 × g for 30 min. The pellet was resuspended in 0.8 mL/g wet heart wt of 600 mM KCl and 10 mM Tris maleate (pH 6.8, 4°C) with a Teflon-glass homogenizer, and centrifuged at 40 000 × g for 30 min. The final pellet was resuspended in 20 mM Tris maleate (pH 6.8, 4°C). Protein concentrations were determined by the method of Lowry (14) with BSA as a standard. The SR membranes were frozen rapidly and stored at −70°C. Preliminary experiments demonstrated that there was no independent effect of the anesthetic or freezing the SR membranes on the measurements reported in this study. Additional experiments demonstrated no differences in Ca^{2+} ATPase and uptake rates between SR membranes isolated from hearts from nonpregnant adult rabbits and the pregnant mothers. The methods for membrane preparation for each age group were

optimized by examining Ca²⁺ ATPase and uptake rates *versus* the speed of the homogenization probe, and the initial dilution volume.

The yield of SR membranes was calculated by dividing the mg of SR membranes that were isolated by the wet wt of left ventricular free wall and septum used for the isolation. The fold enrichment of SR membranes that was accomplished by the isolation procedure was determined by dividing the ⁴⁵Ca²⁺ uptake rate (see below) in the isolated SR membranes by the rate in fresh tissue homogenates (15). We estimated the amount of contaminating sarcolemma in SR membranes by calculating the recovery of the total ouabain-sensitive Na⁺,K⁺ ATPase activity (16) in SR membranes as a percentage of the total activity in the homogenate protein. We also compared the ouabain sensitive Na⁺,K⁺ ATPase activities in SR membranes and in isolated rabbit cardiac sarcolemma membranes (17). All reported Na⁺,K⁺ ATPase activities represent the highest values obtained after preincubation of protein with a range of saponin concentrations (18). We estimated the amount of contaminating mitochondria in SR membranes by calculating the recovery of the total azide-sensitive Mg²⁺ ATPase (see below) in SR membranes as a percentage of the total activity in the homogenate protein (19). All assays of SR membrane function reported in this study were performed in 10 mM sodium azide to inhibit the contaminating mitochondrial activity (19).

Measurements of Ca²⁺ pump function. Ca²⁺ -dependent ATPase activity was measured at 30°C with a linked enzyme system of pyruvate kinase-lactate dehydrogenase by continuously monitoring NADH oxidation at 340 nm (20). The reaction was performed in 40 mM Tris maleate (pH 6.8), 100 mM KCl, 10 mM MgCl₂, 10 mM sodium azide, 40 μM ⁴⁵CaCl₂, 2 mM P-enolpyruvate, 400 μM NADH, 8.75 U of pyruvate kinase, 12.75 U of lactate dehydrogenase, 1 mM Na₂ATP, and 20 μg of SR protein/mL, in the presence and absence of 2 mM EGTA. The difference in the ATP-dependent activity measured in the presence and absence of EGTA was calculated as the Ca²⁺ ATPase activity. The ATP-dependent activity measured in EGTA is reported as the Mg²⁺ ATPase activity.

ATP-dependent, oxalate-supported ⁴⁵Ca²⁺ uptake was measured at 30°C with a vacuum filtration technique (0.45-μm Millipore filters, type HAWP 025 00). The reaction was performed in the conditions used for Ca²⁺ ATPase activity except for the addition of 5 mM sodium oxalate and the exclusion of NADH. Timed aliquots were rapidly filtered before and after the reactions were initiated by the addition of 1 mM Na₂ATP. Each filtered aliquot was washed immediately with 5 mL of 40 mM Tris maleate, 100 mM KCl (pH 6.8, 4°C), and counted in a liquid scintillation counter. The specific radioactivity was calculated by subtracting the nonspecific binding to filters and protein before the addition of ATP from the total radioactivity after the reaction was initiated. Uptake rates were determined by linear regression.

Estimation of active and total Ca²⁺ pump protein content. The content of active Ca²⁺ pump protein in SR membranes was estimated by measuring the Ca²⁺-dependent steady state phosphoenzyme content of cardiac SR membranes with vacuum filtration. The reaction was performed on ice in 10 mM 1,4-piperazine(ethanesulfonic acid) (pH 6.8), 100 mM KCl, 5 mM MgCl₂, 10 mM sodium azide, 40 μM ⁴⁵CaCl₂, and 50 μg protein/mL in the presence and absence of 2 mM EGTA and 5 mM nonradioactive ATP. The reaction was initiated with 50 μM [³²P] ATP, and quenched on ice after 10 s with 0.4 M HClO₄, 20 mM H₃PO₄. The quenched reaction was filtered (Whatman GF-C) and washed with 50 mL of ice-cold 125 mM HClO₄ and 20 mM H₃PO₄. The validity of the filtration technique was determined by acid pH PAGE of the quenched reaction products, with autoradiographic detection of the phosphoenzyme (21).

The content of total Ca²⁺ pump proteins in SR membranes was estimated by immunodetection after electrophoretic separation of the protein. Electrophoresis of 50 μg of SR protein/lane with SDS-PAGE was performed in 7.5% polyacrylamide

slab gels prepared according to Laemmli (22). The SR protein was used for Western transfer (23) to nylon membranes. The membranes were probed with an MAb to the canine cardiac Ca²⁺ pump, which was a generous gift from Dr. Larry Jones. Immunoreactive Ca²⁺ pump protein was exposed to horseradish peroxidase-labeled goat anti-mouse IgG (Pierce Labs, Rockford, IL), which was detected with 4-chloronapthol (Kirkegaard and Perry Labs, Gaithersburg, MD). The results were quantified with laser densitometry. We also used Coomassie brilliant blue staining of 5–15% gradient SDS-PAGE slab gels to detect the SR protein profile in the different isolations.

Isolation and analysis of RNA. We isolated RNA from hearts with the quanididium/phenol/chloroform solvent extraction method (RNAZOLᴮ, Biotecx Labs, Houston, TX). RNA samples were isolated at the same time from 29-d gestation fetal and maternal hearts that had been rapidly frozen at −80°C.

Northern analyses were performed by separating 20 μg of RNA per lane in 1% agarose formaldehyde denaturing gels, with capillary transfer of the RNA to nitrocellulose membranes (24). The membranes were baked at 80°C for 2 h, prehybridized in 20 mL of 50% formamide, 0.8 M NaCl, 50 mM Na₂HPO₄, 1 mM EDTA, 0.5 × Denhardt's solution (24), and 250 μg/mL herring sperm DNA at 55°C for 3 h, and hybridized overnight in 10 mL of fresh prehybridization solution to which we added 3 × 10⁷ cpm of a [³²P]Ca²⁺ pump cRNA probe (Riboprobe, Promega, Madison, WI). The membranes were washed in 0.1 × sodium chloride, sodium citrate and 0.1% SDS at 68°C three times for 20 min, and the probe-RNA hybridization signals were visualized with autoradiography. The membranes were stripped free of [³²P]Ca²⁺ pump cRNA probe by placing the membrane in a boiling solution of 1 × sodium chloride, sodium citrate and 0.1% SDS, and allowing the solution to cool to room temperature. Each membrane was reprobed for constitutively expressed 18S ribosomal RNA with 3 × 10⁷ cpm of [³²P]18S cRNA. The membranes were washed as described above and autoradiographed. The abundances of SR Ca²⁺ pump mRNA and 18S ribosomal RNA were quantified by laser densitometry of the hybridization bands that were detected with autoradiography. We normalized the results in each lane by dividing the Ca²⁺ pump mRNA content by the 18S ribosomal RNA content (11).

For this project, we received generous gifts of a 677-bp *Eco*RI-*Eco*RI human kidney SR Ca²⁺ pump cDNA (bp +772 to +1449 of HK1, see Ref. 25) and a 1.9 kb *Sal*I-*Eco*RI mouse 18S ribosomal cDNA (26). We subcloned each of the cDNA into the Bluescript plasmid (Promega), which we used to generate the cRNA hybridization probes. The human kidney SR Ca²⁺ pump cDNA is an isoform of the cardiac/slow twitch Ca²⁺ pump gene, which, through alternate transcription, codes for sarcoplasmic reticulum or endoplasmic reticulum Ca²⁺ pump mRNA expressed in heart, kidney, gastrointestinal smooth muscle, and brain (10, 27, 28). The alternate splice site is beyond the 3' end of the probe that we used. In preliminary experiments, we determined that both of the probes that we used cross-reacted with rabbit cardiac RNA. The specificity of the probes was determined by comparison of restriction maps with the predictions from sequence data in the Genebank computer program, (Molecular Biology Information Resource, Department of Cell Biology, Baylor College of Medicine, Houston, TX) and by comparison of the sizes of the hybridized Ca²⁺ pump mRNA and ribosomal 18S RNA with published data (11, 26, 29).

Data analysis. All experiments were repeated with four to nine different isolations of SR membranes or RNA. Data are presented as mean ± SEM, or as representative individual experiments when appropriate. When the analyses compared two data groups, we used unpaired *t* tests to detect significant differences ($p < 0.05$). When the analyses compared more than two groups, we used analysis of variance. When the computed F ratio of an analysis of variance exceeded the critical F ratio (at $p = 0.05$),

the Student-Newman-Keuls test was used to identify the groups that were significantly different.

RESULTS

Relative purity of SR membrane preparations. The yields of rabbit cardiac SR membranes were significantly lower in the fetuses and newborns than in the adults (Table 1). In contrast, there were no significant differences among the three age groups in the fold enrichment of the SR membranes by the isolation procedure, or in the ouabain-sensitive Na^+,K^+ ATPase activity in the isolated SR membranes (Table 1). The ouabain-sensitive Na^+,K^+ ATPase activity in the SR membranes was typically less than 2% of the activity in isolated rabbit cardiac sarcolemma membranes (Table 1). There also were no significant differences among the three age groups in the contamination of the SR membranes with sarcolemma and mitochondria. Compared with the homogenates, the percentages of recovery of sarcolemma and mitochondrial marker activities were less than 2 and 4%, respectively (Table 1). All reactions were performed in 10 mM sodium azide to inhibit the residual mitochondrial activity (19).

In agreement with previously published data (8), the rabbit cardiac SR membranes (Table 2) had a higher Mg^{2+} ATPase rate than typically is observed in isolated canine cardiac SR membranes (30). We found that the Mg^{2+} and Ca^{2+} ATPase activities of the adult microsomes did not migrate together through a sucrose gradient (31). The Mg^{2+} ATPase activity preferentially sedimented at the interface of 24 and 30% sucrose, whereas the Ca^{2+} ATPase activity localized to the interface of 30 and 50% (data not shown). Similar results with chicken and rabbit skeletal muscle membranes have suggested that this physically distinct Mg^{2+} ATPase activity is a function of a t-tubular protein (32–34).

SR Ca^{2+} pump protein function and content. The Ca^{2+} ATPase- and oxalate-supported Ca^{2+} uptake rates in isolated rabbit cardiac SR membranes were significantly lower in the fetuses and newborns than in the adults (Table 2). The Ca^{2+} uptake rates in the cardiac homogenates showed a similar significant trend (Table 2). There were no developmental differences in the percentage of increase in the Ca^{2+} ATPase rates in the presence of the Ca^{2+} ionophore A23187 (Table 2), or in the sensitivity of SR Ca^{2+} uptake and ATPase to Ca^{2+}, ATP, pH, and temperature (data not shown). Ca^{2+} uptake and ATPase rates were maximally activated at a free Ca^{2+} concentration of 10 μM or better in all age groups (data not shown).

The number of functional SR Ca^{2+} pumps in isolated SR membranes, as estimated by the steady state Ca^{2+}-dependent phosphoenzyme content, measured with a filtration assay, was lower in the fetus compared with the adult (Table 2; fetal/adult ratio = 0.48 ± 0.05). The validity of the filtration technique was demonstrated by autoradiography after separation of the reaction

products with acid pH PAGE. The major protein that is phosphorylated is the 105-kd CaATPase pump (Fig. 1). Thus, the developmental differences demonstrated in the filtration assay are related to differences in the number of active Ca^{2+} pumps, rather than to phosphorylation of other proteins. The Ca^{2+} dependence of the pump also was demonstrated by the absence of phosphorylated CaATPase when the reaction is performed in the absence of Ca^{2+} (Fig. 1, *lanes 2* and *4*).

The content of total SR Ca^{2+} pumps in isolated SR membranes, as estimated by immunoblotting techniques, was significantly lower in the fetus compared with the adult (Fig. 2; fetal/adult ratio = 0.37 ± 0.05 for n = 4; p < 0.01). These results correlate closely with the estimates of sarcoplasmic reticulum Ca^{2+} pump protein detected by Coomassie blue staining, which were roughly 15 and 25% of the total protein in the fetal and adult sarcoplasmic reticulum membranes, respectively (data not shown).

SR CaATPase mRNA content. We estimated the content of cardiac CaATPase mRNA using Northern analysis with a 677-bp *EcoRI-EcoRI* cRNA probe (Riboprobe) from human CaATPase cDNA (25). The location of the CaATPase message (Fig. 3) is consistent with the predicted 3-kb mRNA size and with previously published data (10, 29). Each membrane was reprobed for the constitutively expressed 18S ribosomal RNA with a 1.9-kb *SalI-EcoRI* mouse 18S cRNA probe, and we normalized CaATPase mRNA expression for 18S mRNA expression with laser densitometry. With this method, we found that the content of CaATPase mRNA was lower in the fetal heart than in the adult (Fig. 3; fetal/adult CaATPase mRNA ratio = 0.39 ± 0.06 for n = 8, p < 0.01). The fetal/adult ratios for the number of active Ca^{2+} pumps, the content of total Ca^{2+} pump protein, and the Ca^{2+} pump mRNA content were not significantly different (Fig. 4).

DISCUSSION

Several previous studies have demonstrated that Ca^{2+} uptake and ATPase rates are slower in fetal and newborn rabbits and sheep compared with adults of the same species (5, 7–8). Our study confirms the slower Ca^{2+} transport function in the developing rabbit heart. The parallel results for Ca^{2+} uptake rates in the cardiac homogenates (Table 2) indicate that the results in the isolated SR membranes are representative of the *in vivo* physiology, and are not an artifact of the isolation procedure.

Ca^{2+} uptake and ATPase rates in isolated SR membranes are the result of several factors, including membrane leakiness, contamination by other organelles, the sensitivity of the Ca^{2+} pump to Ca^{2+}, ATP, pH, and temperature, the effect of several additional SR proteins such as phospholamban, and the content of active Ca^{2+} pumps. There were no significant differences in the percentage of increase in the Ca^{2+} ATPase rates in the presence

Table 1. *Relative purity of SR membrane preparations**

	Fetus	Newborn	Adult
Yield (mg/g)	0.24 ± 0.05†	0.32 ± 0.04†	0.48 ± 0.06
Fold enrichment of SR membranes	21 ± 2	20 ± 3	18 ± 2
Contamination of SR membranes (% recovery)			
Na⁺,K⁺ ATPase (sarcolemma)	0.8 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Azide-sensitive Mg^{2+} ATPase (mitochondria)	2.9 ± 0.4	3.4 ± 0.6	2.7 ± 0.3
Na⁺,K⁺ ATPase activity			
SR membranes	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
SL membranes	1.02 ± 0.04†	ND	2.31 ± 0.03

* Values represent the mean ± SEM from four to eight different preparations. SL, sarcolemma; ND, not done. The variables were measured and calculated as in Materials and Methods. We estimated the amount of contaminating sarcolemma in SR membranes by calculating the recovery of the total ouabain-sensitive Na⁺,K⁺ ATPase activity in SR membranes as a % of the total activity in the homogenate protein. We estimated the amount of contaminating mitochondria in SR membranes by calculating the recovery of the total azide-sensitive Mg^{2+} ATPase in SR membranes as a % of the total activity in the homogenate protein. Na⁺,K⁺ ATPase activity = ouabain-sensitive rate, in μmol/mg/min. All Na⁺,K⁺ ATPase activities represent the highest values obtained after preincubation of protein with a range of saponin concentrations.

† Significantly different from adults at p < 0.01.

Table 2. *Developmental changes in SR calcium transport function**

	Fetus	Newborn	Adult
ATPase rate			
Mg^{2+}	$97 \pm 9\dagger$	$128 \pm 12\dagger$	181 ± 16
Ca^{2+}	$147 \pm 13\dagger$	$191 \pm 18\dagger$	275 ± 22
Ca^{2+}, 2 μM A23187	$332 \pm 28\dagger$	$434 \pm 37\dagger$	559 ± 52
% increase, A23187	122 ± 11	125 ± 17	114 ± 18
Ca^{2+} uptake rate			
SR membranes	$45 \pm 8\dagger\ddagger$	$143 \pm 13\dagger$	270 ± 24
Cardiac homogenates	$2 \pm 0.4\dagger\ddagger$	$7 \pm 1\dagger$	15 ± 2
Phosphoenzyme content	$0.40 \pm 0.05\dagger$	ND	0.81 ± 0.07

* Values represent the mean ± SEM from five to nine different preparations. ND, not done. The variables were measured as in Materials and Methods. The rates and phosphoenzyme content were measured in nmol/mg/min and nmol/mg, respectively.

† Significantly different from adults at $p < 0.01$.

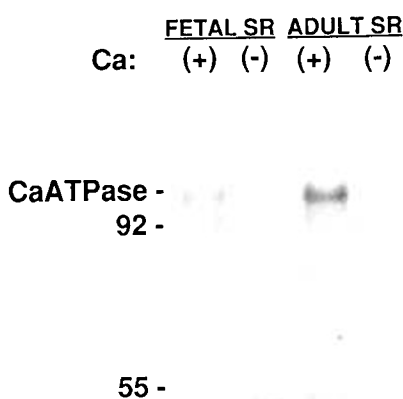‡ Fetuses significantly different from newborns at $p < 0.01$

Fig. 1. The relative contents of active fetal and adult sarcoplasmic reticulum Ca^{2+} pumps. Isolated SR membranes were phosphorylated and the reaction was quenched as described in Materials and Methods. The reaction products were separated by acid pH PAGE, with autoradiographic detection of the steady state phosphoenzyme. *CaATPase*, Ca^{2+} pump of the SR. The numbers at the edge of the figure represent molecular weight markers, in kd.

Fig. 2. The relative contents of total immunoreactive fetal and adult SR Ca^{2+} pumps. The content of total Ca^{2+} pump protein in SR membranes was estimated by immunodetection after electrophoretic separation of the protein and transfer to a nylon membrane, as described in Materials and Methods. The membrane was probed with an MAb to the canine SR cardiac Ca^{2+} pump. Immunoreactive Ca^{2+} pump protein was exposed to horseradish peroxidase-labeled goat anti-mouse IgG, which was detected with 4-chloronapthol. *CaATPase*, Ca^{2+} pump of the SR. The numbers at the edge of the figure represent molecular weight markers, in kd. F and A indicate lanes of fetal and adult SR protein, respectively.

Fig. 3. The relative contents of fetal and adult sarcoplasmic reticulum Ca^{2+} pump mRNA. Northern analysis was performed as described in Materials and Methods with a [³²P]Ca^{2+} pump cRNA probe, using a 677-bp EcoRI-EcoRI human kidney SR Ca^{2+} pump cDNA. The human kidney SR Ca^{2+} pump cDNA is an isoform of the cardiac/slow twitch Ca^{2+} pump gene, which is alternately transcribed beyond the 3′ end of the probe. *CaATPase*, Ca^{2+} pump of the sarcoplasmic reticulum. The numbers at the edge of the figure represent the positions of 28S and 18S RNA, as determined with ethidium bromide staining. F and A indicate lanes of fetal and adult RNA, respectively.

of the Ca^{2+} ionophore A23187 (Table 2), which suggests that the developmental differences in SR membrane Ca^{2+} transport function did not result from artifactual differences in membrane leakiness. Our results also indicate that the SR membranes were not contaminated differentially with membranes from sarcolemma or mitochondria (Table 1). In agreement with previous studies in sheep, we found that the developmental differences in SR Ca^{2+} uptake and ATPase in rabbits were not the result of differences in the sensitivity to Ca^{2+}, ATP, pH, or temperature (5, 7). We did not attempt to estimate phospholamban content in rabbits. However, the lower phospholamban content that has been demonstrated in fetal sheep (5) could not account for the slower Ca^{2+} transport function because phospholamban acts as a suppressor of SR Ca^{2+} pump activity (4, 35, 36). Thus, the slower Ca^{2+} transport function in the fetal heart must be from other factors.

We found that a great deal of the slower cardiac SR Ca^{2+} transport function in the fetus can be attributed to the lower
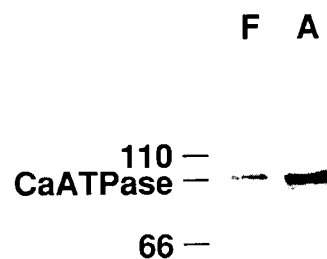
Ca^{2+} pump protein content (Table 2 and Figs. 1 and 2). This was similarly evident from our independent assays of active Ca^{2+} pumps and total immunoreactive pumps. These results appear to exclude the alternate possibility that the fetus could have a similar content of total pump proteins, many of which are inactive. Our results are generally supported by similar results for active and immunoreactive Ca^{2+} pumps in the developing sheep heart (5, 7), as well as the previous morphometric demonstrations of lower SR membrane contents in the developing rabbit heart (6, 37).

We also found that the cardiac SR Ca^{2+} pump mRNA content was lower in the fetal rabbit heart compared with the adult (Fig. 4). The lower Ca^{2+} pump mRNA content in the fetus and the
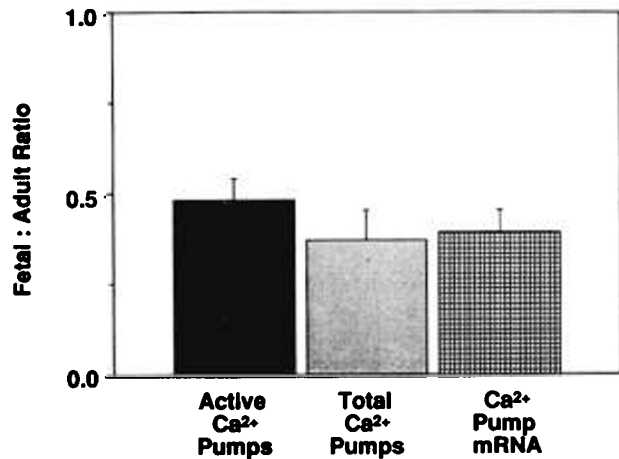
Fig. 4. The fetal/adult ratios of active Ca^{2+} pumps, total Ca^{2+} pumps, and Ca^{2+} pump mRNA contents. The measurements of the contents of active Ca^{2+} pumps, total Ca^{2+} pumps, and Ca^{2+} pump mRNA were performed as described in Materials and Methods. There were no significant differences among the three ratios.

similar fetal/adult ratios of Ca^{2+} pump protein and mRNA content suggest that a considerable part of the slower Ca^{2+} transport function in the fetal SR membranes can be related to transcriptional or posttranscriptional developmental regulation rather than to posttranslational regulation. Our Ca^{2+} pump mRNA data are in agreement with the data of Komuro *et al.* (12) in the developing rat heart. However, Komuro *et al.* (12) found no significant differences in Ca^{2+} pump protein content estimated with Coomassie blue staining of SDS-PAGE gels or in Ca^{2+} uptake rates in isolated SR membranes. This apparent discrepancy may result from differences between species, or from other factors such as the difficulty in isolating SR membranes from rat hearts (38).

Our results indicate that a great deal of the slower sarcoplasmic reticulum Ca^{2+} uptake and ATPase rates in the fetal rabbit heart can be related to lower Ca^{2+} pump mRNA and protein contents. It is evident that transcriptional and/or posttranscriptional regulation of the SR Ca^{2+} pump gene may form an important part of the subcellular basis of the perinatal change in mammalian cardiac relaxation. However, these data do not exclude additional regulatory mechanisms. The recent demonstration of increased Na^+-Ca^{2+} exchanger activity and immunoreactive protein content in the fetal rabbit heart compared with the adult (39) raises the possibility that the slower cardiac relaxation in the fetus may relate to a dependency on alternate mechanisms of Ca^{2+} egress from the cytosol (2). This is in apparent contrast with other muscle-specific proteins, such as myosin, troponin T, and troponin I, which are additionally regulated by isoform switching within a gene family (40–42).

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Announcement

4th International Meeting on Trace Elements in Medicine and Biology

The 4th International Meeting on Trace Elements in Medicine and Biology, "Trace Elements and Free Radicals in Oxidative Diseases," will be held in Chamonix, France, April 5–9, 1993. The meeting is organized by the Society for Free Radical Research, the Société Francophone d'Etude et de Recherche sur les Elements Trace Essentiels, and the Club d'Etude sur les Radicaux Libres. *For registration information, contact:* Professeur Alain Favier or Madame Arlette Alcaraz, Laboratoire de Biochimie C, Hôpital A. Michallon, B.P. 217X, 38043 Grenoble, Cédex 09, France, phone (33) 76 76 54 07; FAX (33) 76 42 66 44.