Expression of adenine nucleotide translocator parallels maturation of respiratory control in heart in vivo

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Portman, Michael A., Yun Xiao, Ying Song, and Xue-Han Ning. Expression of adenine nucleotide translocator parallels maturation of respiratory control in heart in vivo. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H1977–H1983, 1997.—Changes in the relationship between myocardial high-energy phosphates and oxygen consumption in vivo occur during development, implying that the mode of respiratory control undergoes maturation. We hypothesized that these maturational changes in sheep heart are paralleled by alterations in the adenine nucleotide translocator (ANT), which are in turn related to changes in the expression of this gene. Increases in myocardial oxygen consumption ($M\dot{V}O_2$) were induced by epinephrine infusion in newborn (0–32 h, $n = 6$) and mature sheep (30–32 days, $n = 6$), and high-energy phosphates were monitored with ^{31}P nuclear magnetic resonance. Western blot analyses for the ANT_1 and the β-subunit of F_1-adenosinetriphosphatase (ATPase) were performed in these hearts and additional ($n = 9$ total per group) as well as in fetal hearts (130–132 days of gestation, $n = 5$). Northern blot analyses were performed to assess for changes in steady-state RNA transcripts for these two genes. Kinetic analyses for the ^{31}P spectra data revealed that the ADP-$M\dot{V}O_2$ relationship for the newborns conformed to a Michaelis-Menten model but that the mature data did not conform to first- or second-order kinetic control of respiration through ANT. Maturation from fetal to mature was accompanied by a 2.5-fold increase in ANT protein (by Western blot), with no detectable change in β-F_1-ATPase. Northern blot data show that steady-state mRNA levels for ANT and β-F_1-ATPase increased ~2.5-fold from fetal to mature. These data indicate that *1*) respiratory control pattern in the newborn is consistent with a kinetic type regulation through ANT, *2*) maturational decreases in control through ANT are paralleled by specific increases in ANT content, and *3*) regulation of these changes in ANT may be related to increases in steady-state transcript levels for its gene.

myocardial oxygen consumption; mitochondria; sheep; adenosine 5′-triphosphate; adenosinetriphosphatase; oxidative phosphorylation

MITOCHONDRIAL ATP synthesis is tightly coupled to cytosolic ATP utilization in both developing myocardium and the mature heart (18, 24). However, the mode of signal transduction between these two processes appears to change as a function of development. Previous work performed in vitro has generally implied that mitochondrial oxidative phosphorylation responds to increases in cytosolic ADP in a hyperbolic relation emulating a Michaelis-Menten mechanism (15, 20). Studies in mature myocardium in vivo, however, have demonstrated that substantial increases in oxygen consumption are accompanied by minimal change in ADP (18, 24). Thus signal transduction in mature myocardium does not approximate first-order kinetics proposed from isolated mitochondrial studies. In contrast, in newborn myocardium, the relation of oxidative phosphorylation and ADP does appear to follow a simple Michaelis-Menten pattern (23). Alternatively, such increases in ADP during elevated rates of myocardial work in the newborn may instead reflect a decrease in sensitivity to ADP, and more elevated levels are required to drive respiration. Recently, second-order or greater kinetics have been proposed for respiratory control, implying that mature myocardium is exquisitely sensitive to ADP (17). This signal transduction pattern is consistent with a substantial level of control through the adenine nucleotide translocator (ANT), the protein carrier responsible for mitochondrial membrane ADP/ATP exchange. Accordingly, alterations in ADP sensitivity could be due to changes in the apparent number of cooperative ANT binding sites.

Recent work has shown that newborn tissues including myocardium are deficient in ANT sites compared with mature tissues (28). In this study, we have proposed that maturational changes in the relation between ADP and oxidative phosphorylation in vivo are also related to mitochondrial biogenesis with changes in ANT. Furthermore, we postulate that maturation is accompanied by changes in expression of the gene controlling this protein. We have taken a novel approach of study, by which these relations studied in a physiological model in vivo were compared with corresponding protein expression assessed by Western blot, and gene expression assessed through steady-state mRNA levels demonstrated on Northern blots. The model of study is the sheep, in which high-energy phosphate metabolism has been previously outlined during both aerobic and anaerobic conditions in vivo (24, 26).

METHODS

Animal preparation. Animals used in this study were handled in accordance with institutional and National Institutes of Health animal care and use guidelines. Newborn sheep were 0–32 h old, and mature sheep were 30–32 days old. Sheep were sedated with an intramuscular injection of 10 mg/kg ketamine and 0.2–0.4 mg/kg xylazine, intubated, and then ventilated (C-900 pediatric ventilator, Siemens, Schaumberg, IL) with room air and oxygen followed by an intravenous dose of α-chloralose (40 mg/kg). Femoral arterial cannulation was performed for monitoring systemic blood pressure and sampling blood. Arterial pH was maintained between 7.35 and 7.45 by adjustment of ventilatory tidal volume and correction of metabolic acidosis with sodium bicarbonate infusion. After median sternotomy, a coronary sinus-to-superior vena caval shunt was placed as described previously for measurement of coronary sinus flow and myocardial oxygen consumption (24). Platinum-tipped pacing electrodes

were sutured to the right atrial appendage. A 2-cm-diameter nuclear magnetic resonance (NMR) surface coil was sutured to the left ventricular apex. The thoracotomy opening was sealed with plastic wrap to prevent water loss. The sheep, wrapped in a water circulating heating blanket that maintained body temperature at ~38°C, was placed in the 6-cm clear bore of the 4.7-T chemical shift imaging system. The surface coil was positioned at the magnetic center of the system. Blood pressure and coronary sinus flow were recorded on hard copy and to a Macintosh laptop computer equipped with Biotech data-acquisition software.

NMR measurements. After the sheep were transferred into the magnet, the surface coil was tuned to 81 MHz and matched to 50 Ω. NMR data were collected with a General Electric (Fremont, CA) spectrometer using resident software. Shimming on the ^{1}H free induction decay at 200 MHz and acquisition of ^{31}P spectra were performed as previously described, employing cardiac gating (24). The interpulse delay was ~2 s, and the pulse width optimized for the phosphocreatine (PCr) signal, 20–40 μs. All spectra were obtained with a simple one-pulse sequence. Data were acquired with a 5,000-Hz sweep width and 2,048 data points. Sixteen spectra were stored in data-acquisition blocks, and four blocks were averaged for analyses. All spectra were analyzed using a least-squares fit program as well as integration. Fully relaxed spectra were obtained before data acquisition and used for analyses and correction for saturation. Intracellular pH was determined from the chemical shift difference P_i vs. PCr as previously described (24).

Protocol. After stabilization, baseline data were obtained over 8 min. This was followed by epinephrine infusion beginning at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ and titrated upward until a doubling of the mean rate-pressure product was obtained. Data were then acquired for 8 min, and the infusion was increased until a tripling of the mean rate-pressure product was obtained. Epinephrine was decreased and discontinued after 10 min, and 8 min of baseline data were obtained. Hemodynamic data were recorded throughout, and blood sampling was performed at the 4th min. After the protocol was completed, the heart was rapidly excised, and tissue was removed for Northern and Western blotting studies. Additional tissue was obtained from two newborns, which did not undergo the protocol, and from five fetal lambs (130–132 days of gestation) obtained from cesarean section.

RNA isolation. After excess fat and adhering connective tissues were removed, the left ventricular wall was quickly blotted dry, frozen in liquid nitrogen, and stored at −80°C. An aliquot (200 mg) of the frozen tissue was pulverized and homogenized, and total RNA was extracted with a RNA isolation kit (Ambion, Austin, TX). RNA samples were tested by ultraviolet absorption ratio A_{260}/A_{280} for purity and concentration. Values for A_{260}/A_{280} were >1.8 for all RNA extraction. The quality and concentration of the RNA samples were further confirmed by electrophoresis on denatured 1% agarose gels.

Northern blot analysis. RNA (15 μg) was denatured, electrophoresed into a 1% formaldehyde agarose gel, transferred to a nitrocellulose transfer membrane (Micron Separations, Westboro, MA), and cross-linked to the membrane with short-wave ultraviolet light. The prehybridizing and hybridizing solutions contained 50% formamide, 1× Denhardt's solution, 6× saline-sodium phosphate-EDTA, and 1% sodium dodecyl sulfate (SDS). cDNA probes were labeled with [^{32}P]dCTP by random primer extension (Prime-It II, Stratagene, La Jolla, CA) and added to the hybridizing solution. Hybridization was performed at 42°C for 18 h. The blots were then washed several times with a final wash in 0.1× standard sodium citrate (SSC) and 0.1% SDS at 65°C. The relative content of mRNAs was evaluated by scanning densitometry using a PhosphorImager model 400S and ImageQuant quantitation software (Molecular Dynamics, Sunnyvalve, CA). ANT_1 and β-F_1-adenosinetriphosphatase (ATPase) mRNA loading was normalized to 28S ribosomal RNA band. ANT_1 mRNA levels were detected using a 1.4-kb insert cDNA cloned from the human skeletal muscle [American Type Culture Collection (ATCC), Rockville, MD]. β-F_1-ATPase mRNA levels were detected using a 1.8-kb insert cDNA cloned from human HeLa cell line (ATCC). To compare different mRNA levels in the same myocardial sample, aliquots of 15 μg of total RNA from the myocardium were analyzed by means of reprobing the membrane with 28S, ANT_1, and β-F_1-ATPase cDNA probes.

Western blot analysis. Frozen tissue samples were homogenized in boiling 2% SDS extract solution, and the homogenates were centrifuged at 2,000 *g*. Aliquots of the resulting supernatants were fractionated in SDS, 12.5% polyacrylamide gels, transferred to polyvinylidene difluoride membranes (Millipore), and Western blotted with antisera developed in rabbit to purified rat liver mitochondrial β-F_1-ATPase (22) or rabbit antisera to rat heart adenine nucleotide translocase (5). The immunoreactive protein was visualized with goat anti-rabbit immunoglobulin G-peroxidase conjugate. All blots were developed with the enhanced chemiluminescence system (Amersham). Intensity of the ANT or β-F_1-ATPase bands was performed with laser densitometric scanning. For standardization purposes, the same amount of protein was run in parallel lanes on an SDS gel. Densitometric scanning revealed no differences in the amount of protein loaded per lane. Quantitation was performed with scanning densitometry.

RESULTS

High-energy phosphates and oxygen consumption. Metabolic data for the mature and newborn groups are summarized in Tables 1 and 2, respectively. The newborns studied in these experiments were a few days younger than those investigated in our previous experiments (24, 25). Pressure-rate product (mean blood pressure × heart rate) is also reported in Tables 1 and

Table 1. *Mature sheep heart metabolic and hemodynamic values during epinephrine infusion*

Level	PCr/ATP	pH_i	ADP	$M\dot{V}O_2$	BP	HR	PRP
Baseline	1.54 ± 0.04	7.14 ± 0.01	0.038 ± 0.003	2.41 ± 0.19	71 ± 3	156 ± 9	11,110 ± 880
1	1.47 ± 0.03	7.12 ± 0.01	0.042 ± 0.003	3.50 ± 0.30*	86 ± 5	211 ± 9*	17,588 ± 1,172†
2	1.44 ± 0.04	7.11 ± 0.02	0.044 ± 0.004	4.88 ± 0.35†	97 ± 6*	274 ± 12†	26,585 ± 1,598†
Recovery	1.53 ± 0.03	7.12 ± 0.01	0.037 ± 0.001	3.73 ± 0.60*	80 ± 6	235 ± 22*	19,307 ± 3,119†

Values are means ± SE. PCr, phosphocreatine; pH_i, intracellular pH; ADP, in mM; $M\dot{V}O_2$, myocardial O_2 consumption in $\mu mol \cdot g^{-1} \cdot min^{-1}$; BP, mean arterial blood pressure in mmHg; HR, heart rate in beats/min; PRP, pressure-rate product (HR × BP) in $beats \cdot min^{-1} \cdot mmHg$. Significance vs. baseline: *$P < 0.01$; †$P < 0.001$.

Table 2. *Newborn sheep heart metabolic and hemodynamic values during epinephrine infusion*

Level	PCr/ATP	pH_i	ADP	$M\dot{V}O_2$	BP	HR	PRP
Baseline	1.32 ± 0.02	7.15 ± 0.01	0.060 ± 0.002	2.73 ± 0.06	50 ± 3	168 ± 9	8,423 ± 749
1	1.04 ± 0.03*	7.13 ± 0.01	0.093 ± 0.005*	4.60 ± 0.14*	72 ± 8*	247 ± 12†	17,475 ± 1,751†
2	0.92 ± 0.02†	7.10 ± 0.01	0.106 ± 0.004†	5.60 ± 0.14†	73 ± 4†	313 ± 10†	23,090 ± 1,776†
Recovery	1.20 ± 0.04	7.10 ± 0.01	0.067 ± 0.004	3.15 ± 0.07*	52 ± 3	218 ± 11†	10,993 ± 818

Values are means ± SE. Significance vs. baseline: *$P < 0.01$; †$P < 0.001$.

2. Similar baseline oxygen consumption rates were present in the two groups. The range and peak oxygen consumption rates induced by epinephrine infusion were also similar. As noted in previous studies (24, 25) the mature sheep do not show any change in PCr/ATP during these twofold or slightly greater increases in oxygen consumption. Although this oxygen consumption increase is fairly mild relative to the range in unsedated lambs (6), it is nevertheless accompanied by substantial and significant decreases in PCr/ATP in newborn lambs ($P < 0.001$ vs. baseline and mature sheep values). Cytosolic ADP concentration calculation in this sheep model has been described in detail previously (24, 25). These values are derived from previous determinations of cytosolic ATP and creatine, which are applied using the creatine kinase reaction and the equilibrium constant published by Lawson and Veech (21). Ingwall et al. (12) have shown that developmental changes in cytosolic ATP and creatine pools do not occur after birth in sheep. PCr/ATP is slightly lower and calculated ADP is slightly higher at baseline in the newborn sheep. This represents a difference from previous studies (24, 25), which demonstrated that a cohort of lambs with age a few days older showed no difference from a mature group. Unlike the mature sheep, these newborns do substantially increase ADP during increases in oxygen consumption. Individual ADP vs. oxygen consumption data are plotted in Fig. 1 for the two groups. Representative spectra from a newborn lamb experiment with difference spectra are plotted in Fig. 2. These data show that corresponding increases in P_i occur during the decreases in PCr, whereas ATP remains stable. Although there is individual variability, intracellular pH does not change significantly during oxygen consumption increases in either group.

Steady-state mRNA levels. Semiquantitative analyses of steady-state mRNA levels for ANT and β-F_1-ATPase were performed with Northern blotting for three groups: fetal ($n = 5$), newborn ($n = 9$), and mature ($n = 9$). A representative Northern blot is demonstrated in Fig. 3. Figure 3 (*left*) shows bands for 28S ribosomal RNA as well as the bands for ANT_1; Fig. 3 (*right*) shows reprobing for β-F_1-ATPase in the same membrane. In many species ANT_1 gene expression in heart and skeletal muscle far exceeds that of the other two ANT genes, ANT_2 and ANT_3. Because these latter genes are weakly expressed in heart, only steady-state transcript levels for ANT_1 were assessed. Similar to bovine heart, two ANT_1 transcripts were observed to produce dual bands at 1.4 and 1.2 kb in sheep heart. Stepien et al. (30) have attributed these two bands to different length 3′-nontranslated sequences generated by the bovine genes' two polyadenylation sites. Relative intensities for each mRNA in the separate age groups are shown in Fig. 4. These data show that, for both ANT_1 and β-F_1-ATPase, there are statistical differences for steady-state mRNA levels between fetal and mature groups. There is an ~2.5-fold increase in steady-state levels of mRNAs for both of these genes during the transition from fetal to the mature age (30 days in this model).

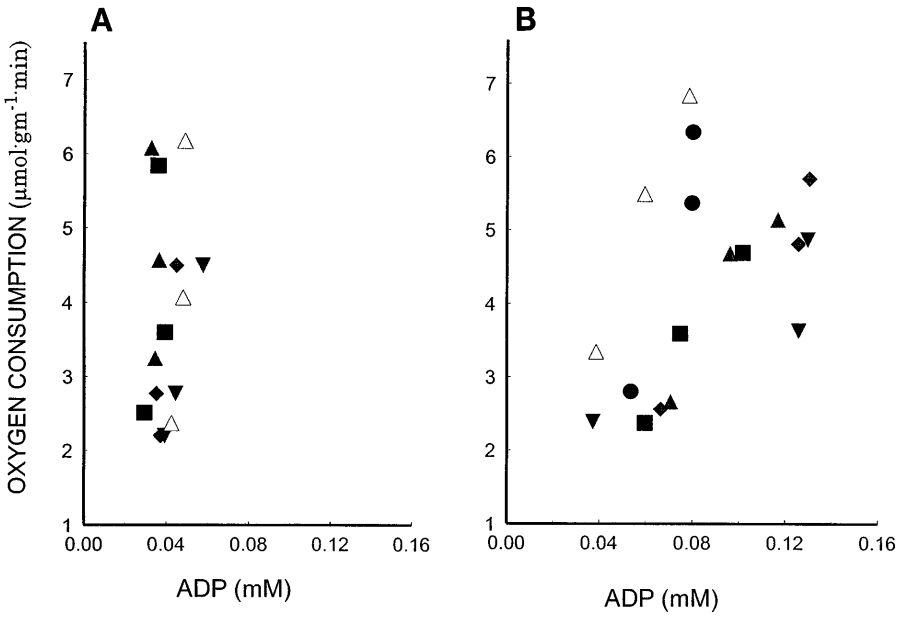

Fig. 1. Myocardial oxygen consumption vs. ADP plotted for individual experiments in mature (*A*) and newborn (*B*) sheep. Each symbol represents a different sheep; $n = 6$.

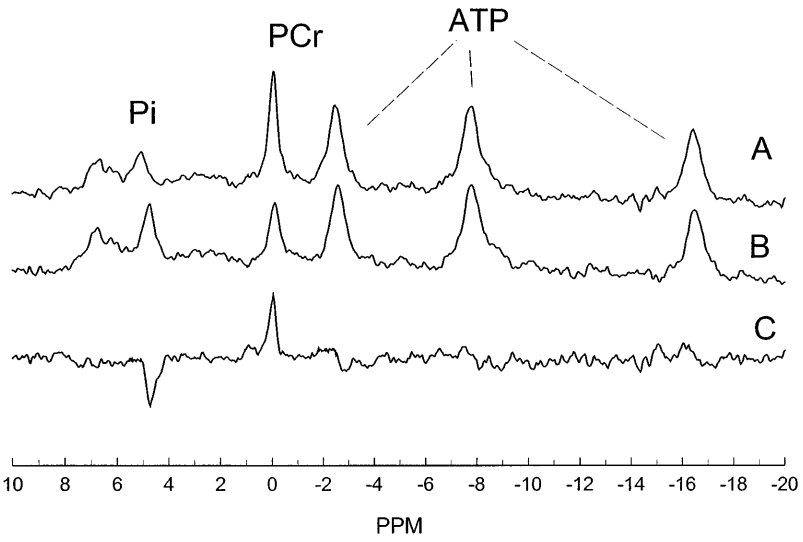

Fig. 2. Representative spectra from a newborn lamb experiment are shown. *A*, baseline; *B*, obtained during a 2-fold increase in myocardial oxygen consumption; *C*, difference spectra (*A* − *B*). Corresponding increase in P_i occurs during phosphocreatine (PCr) decrease. In this particular experiment, there is a slight downfield shift to P_i peak, indicating a drop in intracellular pH (pH_i). However, pH_i did not change significantly in entire experimental group. PPM, parts per million.

These data thus indicate that there is a coordinated increase in these steady-state transcript levels during this maturational period.

Protein levels. Protein levels for ANT and β-F_1-ATPase in heart were assessed semiquantitatively by Western blotting. A representative Western blot is shown in Fig. 5. Relative normalized densitometric intensities are shown in Fig. 6. These data demonstrate a greater than twofold increase in ANT_1 during the maturational transition between the fetal and mature heart. The data imply that increases in this protein's levels occur after the 1st or 2nd day of life. In contrast, there appears to be no change in β-F_1-ATPase after 130 days of gestation in the sheep heart.

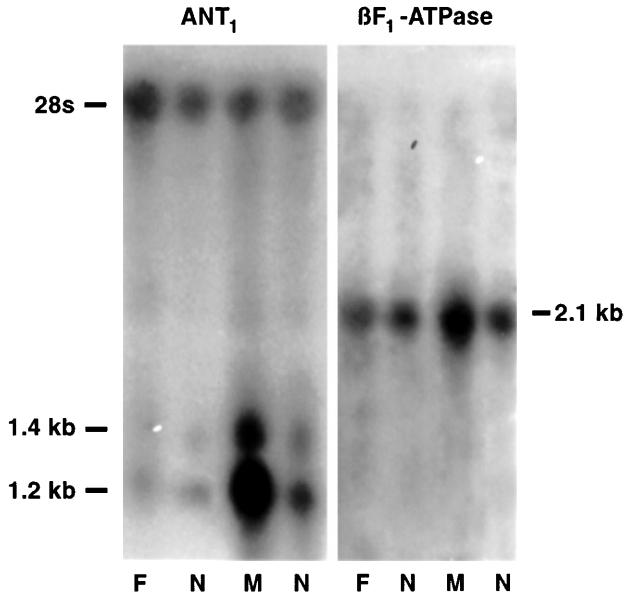

Fig. 3. Representative Northern blot loaded with ventricular myocardium and specific probes as noted. Same blot was reprobed for β-F_1-ATPase. Two adenine nucleotide translocator (ANT_1) bands appear at 1.4 and 1.2 kb. This blot emphasizes increases in ANT_1 and β-F_1-ATPase RNA with maturation. F, fetal; N, newborn (0–32 h); M, mature (30–32 days).

DISCUSSION

Kinetics. The data obtained in these studies are consistent with the hypothesis that ANT participates in regulation of myocardial respiration in the newborn. Possibly, limitations in translocator function are responsible at least in part for observed maturational changes in the relation between oxidative phosphorylation and ADP. The nature of limitations in the translocator is unclear. Investigators have previously assumed that respiratory regulation is dependent on ADP availability at the mitochondrial membrane (15, 16, 20). This concept is consistent with regulation through the translocator, which should follow simple Michaelis-Menten kinetics. However, lack of predicted change in ADP during increases in myocardial oxygen consumption in mature myocardium in vivo has led to conclusions that respiratory control must occur through alternative mechanisms (2, 8, 9), including control through changes in reducing equivalent supply to the respiratory chain as well as through modulation of mitochondrial Ca^{2+} concentration (2, 8, 9). Jeneson et al. (17) stimulated renewed interest in the translocator by fitting data

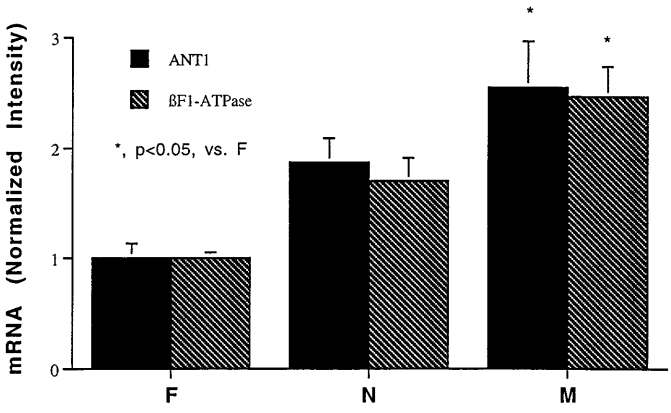

Fig. 4. Relative densitometric intensities for ANT_1 and β-F_1-ATPase are shown for each age groups. Intensities are normalized to 28S band intensity. Significant increase in intensities occur during maturation from fetal to mature.

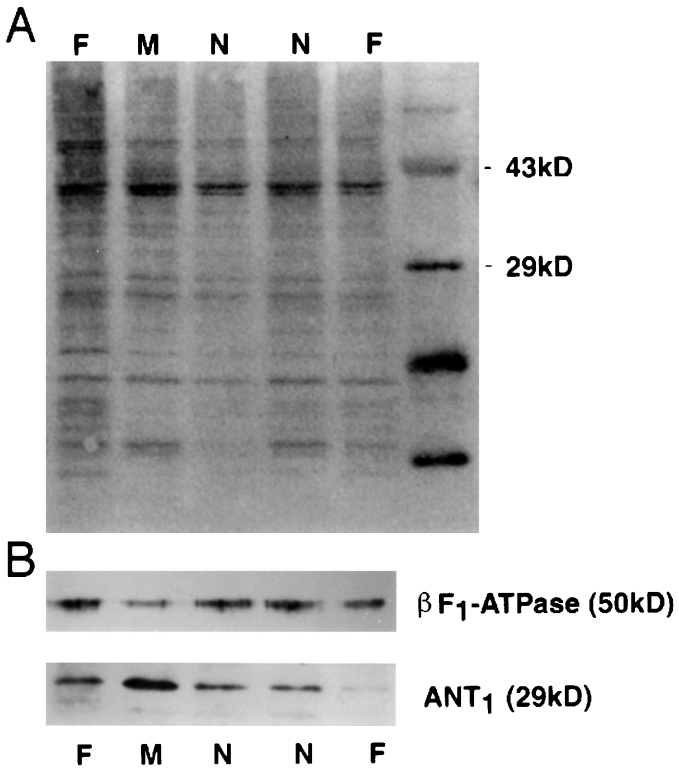

Fig. 5. Representative Western blots assess relative amounts of mitochondrial proteins (ANT_1 and β-F_1-ATPase) in left ventricular myocardium during different developmental states (F, N, and M). Unmarked lane is reference with migration of molecular mass markers as noted. *A*: total protein is similar in all lanes. *B*: bands for ANT_1 and β-F_1-ATPase after immunoprecipitation with specific anitibodies. Substantial increase in ANT_1 protein but not in β-F_1-ATPase occurs with maturation.

obtained from several published diverse experimental preparations, including canine myocardium in vivo (18), to a model employing sensitivity amplification as described by Koshland (19). This ultrasensitivity model demonstrates substantially greater change in reaction velocity (oxidative phosphorylation) over a much narrower range in relative substrate (ADP) concentration than defined by the hyperbolic relationship of Michaelis-Menten kinetics. Increased sensitivity can be due to allosteric type enzyme activation and is consistent with cooperative activation of ANT. Corroboration of the ultrasensitivity model in vivo usually requires data from a wide range of respiratory rates. The model as published used canine data obtained during increases in ADP induced by phenylephrine infusion and possibly due to relative ischemia but not to increases in myocardial oxygen consumption rate. To evaluate sigmoid saturation kinetics over a relatively narrow range of respiratory rates, a graphic representation of the Hill equation can be employed (27). In the form of a straight line, the Hill equation for the relation between ADP and respiratory rate can be adapted as $\log v_i/(V_{max} - v_i) = n\log [S] - \log k'$, where v_i is the myocardial oxygen consumption rate, V_{max} is the maximal oxidative phosphorylation rate (taken as 20 $\mu mol \cdot g^{-1} \cdot min^{-1}$ in sheep; Ref. 6), [S] is ADP concentration, and k' is a complex constant. The equation states that when the substrate (ADP) is low compared with k', the reaction velocity increases as the nth power of the substrate concentration. A plot of log ADP concentration vs. $\log v_i/(V_{max} - v_i)$ yields a straight line where the slope equals n; n is an empirical parameter whose value depends on the number of cooperative binding sites. When $n = 1$, the binding sites act independently of one another; when $n > 1$, the sites are cooperative; and when $n < 1$, the sites are said to exhibit negative cooperativity. The data in this format for the newborn and mature sheep heart experiments are plotted in Fig. 7. Because there is no significant change in ADP with increases in oxygen consumption in the mature heart, the derived line is near vertical and the data within this limited range of respiration do not conform to this model. This would imply that neither ADP nor the translocator plays a role in regulation of myocardial respiration in the normal mature heart even if second-order or greater kinetics are considered. Within the error of the data, the slope obtained from newborn lambs is consistent with Michaelis-Menten kinetics ($n = 1.0$). This implies that altered cooperativity does not explain the maturational differences in the relation between ADP and myocardial oxygen consumption.

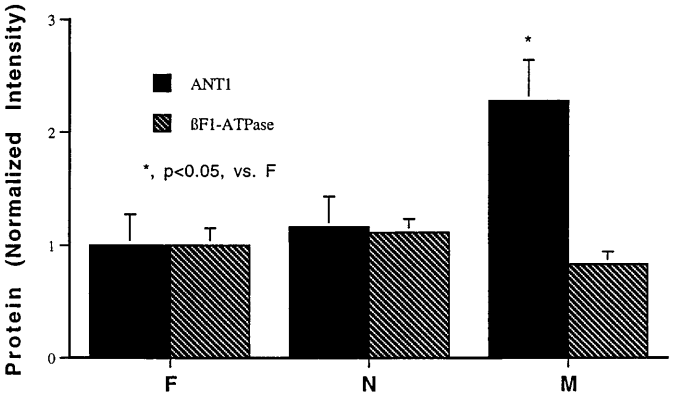

Fig. 6. Normalized densitometric intensities for protein bands from Western blots are shown. These data imply that a greater than twofold increase in ANT_1 protein levels occurs during maturation from fetal to mature. However, no detectable increase in β-F_1-ATPase protein occurs during same period.

Adenine nucleotide translocase protein content. These differences may be due to other factors related to ANT. Changes in ANT activity or protein content in the mitochondrial membrane might be responsible for the observed maturational changes in vivo. Previous work in isolated mitochondria does support the hypothesis that respiratory control through the translocator decreases with maturation (28, 29, 32). Although not a specific focus of their study, Wells et al. (32) demonstrated in isolated sheep mitochondrial preparations that decoupling of oxidative phosphorylation through DNP produces near twofold increases in state III respiratory rates in fetal and newborn mitochondria, with no comparable change occurring in adult mitochondria. This implies that respiration in fetal and newborn mitochondria can be limited or regulated at the phosphorylation level, which consists of two membrane components: mitochondrial ATPase and ANT. More

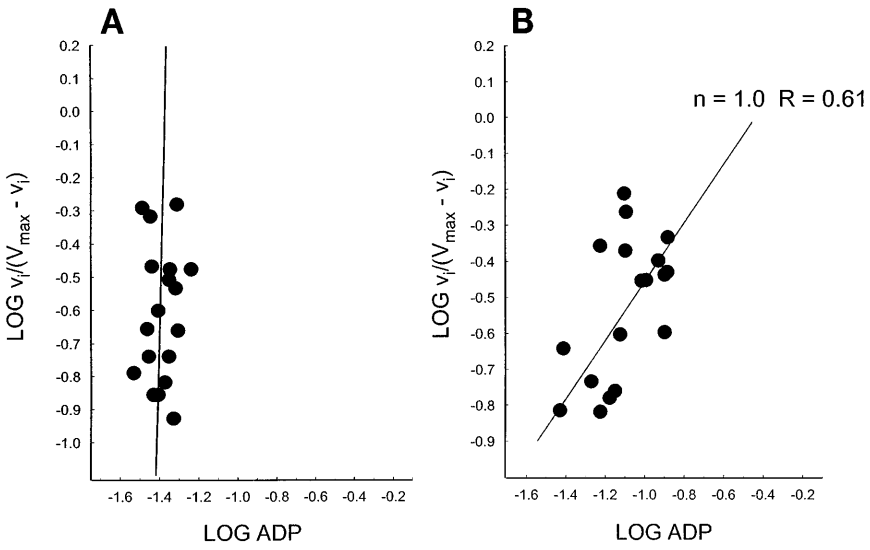

Fig. 7. An analysis using Hill equation is shown for mature (*A*) and newborn (*B*) sheep (see text for details). These data for mature hearts emphasize lack of change in ADP that occurs with increases in oxidative phosphorylation. Coefficient for newborn data (1.0) is consistent with that predicted for Michaelis-Menten or 1st-order kinetics. v_i, Myocardial O_2 consumption rate; V_{max}, maximal oxidative phosphorylation rate.

recently, Shonfeld et al. (29) titrated isolated rat heart mitochondria from different developmental states with the specific adenine nucleotide translocase inhibitor, carboxyatractyloside, during state III respiration. Flux control coefficients estimated from the titration curves suggest that ANT exerts substantial control over respiration in the newborn rat heart mitochondria at state III, which declines progressively to near zero with age. Similar control patterns were demonstrated by the F_1/F_0-ATP synthase (28). The changes in control through the translocator corresponded to ANT activity as well as protein content assessed by Western blot techniques, although age-dependent increases in the matrix-exchangeable adenylate pool also participate. Similarly, the present data show that maturational changes in cellular ANT protein content parallel alterations in the relationship of cytosolic ADP and respiration in sheep heart in vivo. The current data also indicate that the F_1-ATPase component of the F_1/F_0-ATPase reaches adult levels at the fetal stage, thus suggesting that content of this protein does not influence maturational change in respiratory control in the sheep heart after birth. The Western blot data with regard to this particular protein correspond to previous studies which have shown that no changes in the ATPase activity occur after 136 days gestation in sheep (32). The lack of change in β-F_1-ATPase protein also demonstrates that the maturational increase in ANT_1 protein is not just part of a generalized mitochondrial protein increase. Thus the present data obtained in vivo as well as previous studies in vitro support the hypothesis that ANT exerts control over myocardial respiration, which diminishes with age.

Regulation of the expression of the mammalian nuclear-encoded mitochondrial proteins, ANT_1 and the F_1-ATPase, has been described principally in liver (1, 7, 14). Coordinate expression of the genes controlling the two proteins is well established (4, 30). The Northern blot data from our experiments, which show a coordinate increase in steady-state levels of mRNAs for these two proteins in the mature sheep heart, are consistent with these previous studies. Specific regulation of the β-subunit of the mitochondrial F_1-ATPase, which has been used as a reporter protein of nuclear gene activity and as a marker of mitochondrial biogenesis, occurs both at the transcriptional and posttranscriptional levels in liver (13, 14, 22). Uncoordinated changes in RNA and protein levels for the liver β-F_1-ATPase occur during maturation, which may be secondary to tissue-specific activation of translational factors (14). Izquierdo et al. (14) have shown that transcript and protein levels for β-F_1-ATPase are markedly elevated in heart relative to other tissues, a finding implying that regulation is principally exerted at the translational level. It is thus conceivable that altered translational efficiency may be responsible for the disparity between maturational changes in steady-state RNA levels and protein levels for this particular subunit in heart. In contrast, there appears to be a coordinate increase in the mRNA and protein accumulation of ANT_1 in heart. As stated above, this protein accumulation occurs in parallel with changes in respiratory control pattern. This implies that maturation of myocardial respiratory control is induced in part at the transcriptional level, whether related to stability of RNA or new trancriptional products, as well as at the translational level. Control of transcription and/or translation of ANT in vitro has been demonstrated to occur through regulatory factors such as thyroid hormone and oxygen (4, 10, 11, 31), which do change substantially after birth (3).

In summary, these experiments show that regulation of myocardial respiration changes as a function of development in the sheep heart. Changes in the adenine nucleotide translocase protein content parallel maturation of respiratory control. Furthermore, specific increases in steady-state mRNA levels for this protein are associated with both the respiratory control changes and accumulation of the protein. This implies that maturation of myocardial respiratory control may be stimulated at least in part at the level of transcription.

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