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THE ADENINE NUCLEOTIDE TRANSLOCATOR: REGULATION AND FUNCTION DURING MYOCARDIAL DEVELOPMENT AND HYPERTROPHY

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SUMMARY

1. The present review focuses on the adenine nucleotide translocator (ANT), which facilitates exchange of cytosolic ADP for mitochondrial ATP. This protein serves a central role in regulating cellular oxidative capacity.

2. The ANT, a nuclear-encoded mitochondrial protein, is developmentally regulated and, thus, accumulates within the mitochondrial membrane during maturation.

3. Accumulation of ANT parallels changes in kinetics of myocardial respiration determined from ^{31}P magnetic resonance spectroscopy studies.

4. Thyroid hormone modulates developmental transitions in ANT content, as well as respiratory control patterns. These transitions are linked to quantitative ANT changes, not to alterations in functionality at individual exchanger sites.

5. Developmental programming for ANT and parallel alterations in oxidative phosphorylation kinetics are relevant to the heart, which exhibits remodelling in response to pathological processes. Maladaptive hearts exhibiting ANT deficits demonstrate ADP-dependent respiratory kinetics similar to the newborn heart. Thus, ANT deficits and alterations in mitochondrial respiratory function may contribute to the pathogenesis of myocardial remodelling and heart failure.

Key words: adenine nucleotide translocator, energy metabolism, F1-F0-ATPase, mitochondria, oxidative phosphorylation.

INTRODUCTION

The adenine nucleotide translocator (ANT) serves a central role in regulation of energy production in the myocardium. The protein spans the inner mitochondrial membrane and facilitates exchange of cytosolic ADP for mitochondrial ATP (Fig. 1).[1] As the single most abundant protein within mitochondrial membrane, ANT provides a candidate site for convergence of processes or pathways that determine viability of the mitochondrion, as well as the entire myocyte. Thus, this protein has received attention for its participation in respiratory control, pathogenesis of myocardial remodelling and alterations in mitochondrial membrane integrity, which promote apoptosis.[2,3]

Deficits in mitochondrial oxidative capacity and alterations in respiratory kinetics can produce striated muscle dysfunction, where no phenotypic changes in contractile proteins occur.[4] Such data indicate that contractile failure may be caused by primary deficiencies in energy production or storage. In some models of cardiac hypertrophy, which progress to contractile failure, observed respiratory kinetics resemble those apparent in the immature heart.[5,6] These kinetic patterns have been linked to ANT content within the mitochondrial membrane,[7] suggesting that a reiteration of fetal programming for this oxidative phosphorylation gene occurs during heart failure. A review of the changes that occur in respiratory control and regulation of ANT during myocardial maturation seems pertinent to the understanding of these processes as they relate to cardiac remodelling during disease states.

PRINCIPAL FUNCTION OF THE ANT

Exchange of cytosolic ADP for ATP across the mitochondrial membrane represents the principal function for ANT.[2] This process occurs in conjunction with proton translocation through the mitochondrial F1-F0-ATPase and results in phosphorylation of ADP using energy derived from the membrane electrochemical potential.[8,9] The myocardial energy utilizing processes require rapid and unlimited exchange of adenine nucleotides by ANT.[2,10] During increases in cardiac work, signal transduction occurs between the cytosolic energy utilizing sites and oxidative phosphorylation sites within the mitochondria. The heart requires rapid signal transduction between these processes in order to maintain a steady supply of high-energy phosphates and to preserve energy stores. It is probable that several modes of signal communication exist that may vary according to work state.[9,11] Because ANT theoretically limits the rate of ADP supplied to the mitochondria and, hence, ATP to the cytosol, this

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protein can serve as a regulatory site for oxidative phosphorylation during appropriate conditions.

RESPIRATORY KINETICS

The ADP/ATP exchange is similar to enzyme catalysed processes, where reaction rates are controlled by substrate concentration. Thus, ADP drives respiration in a hyperbolic mode emulating typical first-order Michaelis–Menten mechanisms in the simple system consisting of isolated mitochondria *in vitro* (state III).[2,12] The kinetics observed in such experiments conform to theories implicating respiratory control and occur through ANT. Flux control coefficients, which reflect the degree of a reaction's control exerted by an enzyme or protein, can be determined for ANT through carboxyatractyloside titration during state III respiration.[13–15] Paradoxically, experiments using control flux theory, using this specific and non-reversible ADP/ATP exchange inhibitor, indicate that near-zero relative control strength can be attributed to this exchanger in mitochondria isolated from normal adult myocardium. The control strength does relate reciprocally to the maturational state.[13] Specifically, Schoenfeld[13] demonstrated that the newborn rat heart exhibits a relatively high control coefficient (0.39) for ANT, which declines progressively to near 0 with ageing. The maturational change in control coefficient (1.0 indicates that a specific enzyme has complete control) corresponds to change in ANT protein content within the mitochondrial membrane. These findings suggest that changes in respiratory control are caused by accumulation of ANT through development.

Control coefficients and respiratory states are determined using isolated mitochondria suspensions, which can have limited applicability to operative conditions *in vivo*. ^{31}Phosphorous magnetic resonance spectroscopy (MRS) provides a method to determine kinetics of ADP phosphorylation in the intact heart.[16–18] The concentration of ADP cannot be determined by ^{31}P MRS directly due to low ambient cytosolic concentrations. However, [ADP] can be calculated indirectly by such experiments through the creatine kinase equilibrium reaction:

$$[ADP] = [ATP][Cr]/K_{eq}[PCr][H^+]$$

where square brackets indicate concentrations, Cr is creatine, PCr is phosphocreatine and the equilibrium constant K_{eq} is 1.66×10^{-9}, as reported by Veech *et al.*[19]

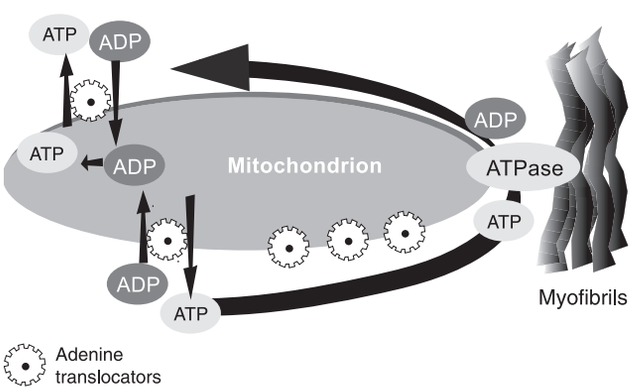

Fig. 1 A schematic representation of the cardiac myocyte. The relationship between the mitochondria and myofibres is illustrated, emphasizing the central role of the adenine nucleotide translocator.

Several investigators have attempted to evaluate respiratory control mechanisms *in vivo* using MRS.[11,16,18,20] If cytosolic [ADP] can be defined at various respiratory rates in an individual heart *in vivo*, then kinetics of oxidative phosphorylation can be compared with models that emulate Michaelis–Menten-type control through ANT. This methodology can define whether changes in respiratory control occur *in vivo* through development. Most studies in mature heart *in vivo* have demonstrated that [ADP] is constant over a moderate range of oxygen consumption rates.[11,16,20] These findings in several mammalian species indicate that ADP and, thus, ANT play negligible roles in regulating oxidative phosphorylation in mature myocardium at moderate workloads. Small changes in ADP have been detected at very high work-states, implying that ANT may control at those higher levels.[11,20,21] Some investigators have theorized that myocardial oxygen consumption responds to very small decreases in [ADP] at the more modest levels of oxidative phosphorylation, which are amplified and more easily detectable at higher work levels.[22] This ultrasensitivity model assumes that ADP binds to ANT cooperatively at multiple sites and stimulates activity allosterically.

Enzymes involved in cooperative binding do not demonstrate classic Michaelis–Menten kinetics.[23] Instead, plots of substrate concentration versus reaction rate yield a sigmoid reaction curve, similar to the haemoglobin–oxygen saturation curve. Such complex relationships would be difficult to define with ^{31}P MRS and cardiac oxygen consumption data. However, these kinetic patterns can be simplified in a graphical representation over a relatively narrow range of respiratory rates by using a straight line function defined by the Hill equation.[23] This function has been used to determine whether myocardial oxygen consumption responds to ADP in a simple or cooperative manner and whether maturational differences in respiratory control occur.[7] The relationship between ADP and respiratory rate is represented as:

$$\log vi/(V_{max}\ vi) = n\log[S]\ \log k'$$

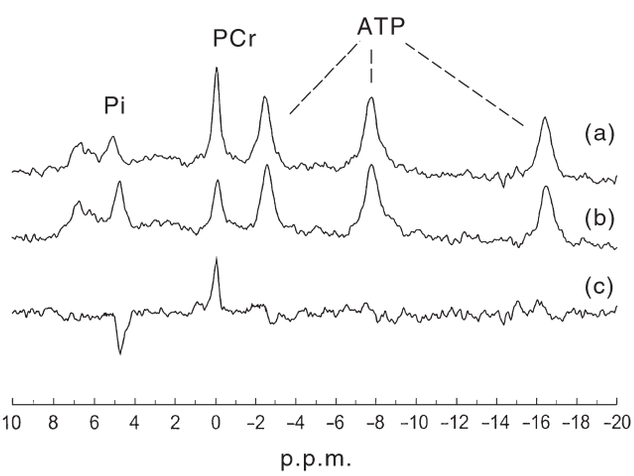

Fig. 2 ^{31}P spectra obtained from the heart of a newborn lamb at age 24 h are illustrated. Spectrum (a) was obtained at baseline, while spectrum (b) shows the effect of an increase in oxygen consumption caused by adrenaline infusion. Spectrum (c) is the difference between (a) and (b). Note the decrease in phosphocreatine (PCr) and the increase in inorganic phosphate (Pi) caused by the increase in oxygen consumption. Reprinted with permission of the American Physiological Society from Portman *et al.*[7]

where vi is the myocardial oxygen consumption rate, V_{max} is the maximal oxidative phosphorylation rate, [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 versus log vi/(Vmax·vi) yields a straight line with slope = n; n is an empirical parameter dependent 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. ^{31}P spectra obtained from the heart of a newborn lamb at age 24 h (Fig. 2) demonstrate changes in phosphocreatine and, thus, ADP that occur during transitions in oxygen consumption rate. No such changes took place in mature sheep heart during similar pertubations. Figure 3 illustrates data obtained from a group of mature and newborn lambs, subjected to analyses using the Hill equation. Because no significant change in [ADP] occurred with increases in oxygen consumption in the mature heart, the derived line is near vertical. The mature heart data did not conform to either Michaelis–Menten or cooperative activation models. Thus, neither ADP nor ANT participate in regulation of myocardial respiration in the normal mature heart *in vivo* within this range of respiration. The slope obtained from newborn lambs is consistent with Michaelis–Menten kinetics (n = 1.0), but not with cooperative binding to ANT. These newborn patterns are consistent with control flux experiments indicating that ANT exercises greater respiratory control in the newborn heart than in the mature.[13] Furthermore, developmental transitions in these kinetic patterns are accompanied by changes in mitochondrial ANT content. Thus, ANT protein accumulation during maturation is conceivably responsible for the transition to a respiratory control mode that does not depend on ADP.

TRANSCRIPTIONAL REGULATION OF ANT

The ANT is a nuclear-encoded protein, subject to regulation at several control levels. Three distinct isoforms (ANT1, ANT2, ANT3) demonstrate tissue specificity and transcript expression responds to cellular demands by variations in the rate of isoform mRNA synthesis and/or stability.[24–26] Although cardiac muscle expresses all three isoforms, ANT1 predominates.[25] Transcriptional regulation of ANT1 occurs in myogenic mouse cells through a muscle-specific positive promoter element, termed OXBOX, and an overlapping tissue ubiquitous region, REBOX.[24,26,27] Binding of factors to the REBOX element is regulated by environmental factors, including the thyroid hormones tri-iodothyronine (T_3) and thyroxin (T_4). The OXBOX element, which is shared by the F1-F0-ATPase β-subunit, appears upstream of the putative transcription initiation site for these oxidative phosphorylation genes. The sharing of this unit provides a mechanism that can provide coordinated regulation of these two mitochondrial membrane proteins at the transcriptional level. Accordingly, cardiac steady state mRNA expression for both these genes increases during developmental transition from fetal to mature stages in mammalian species.[7,28] Although ANT protein expression parallels ANT1 mRNA expression during maturation, coordinate

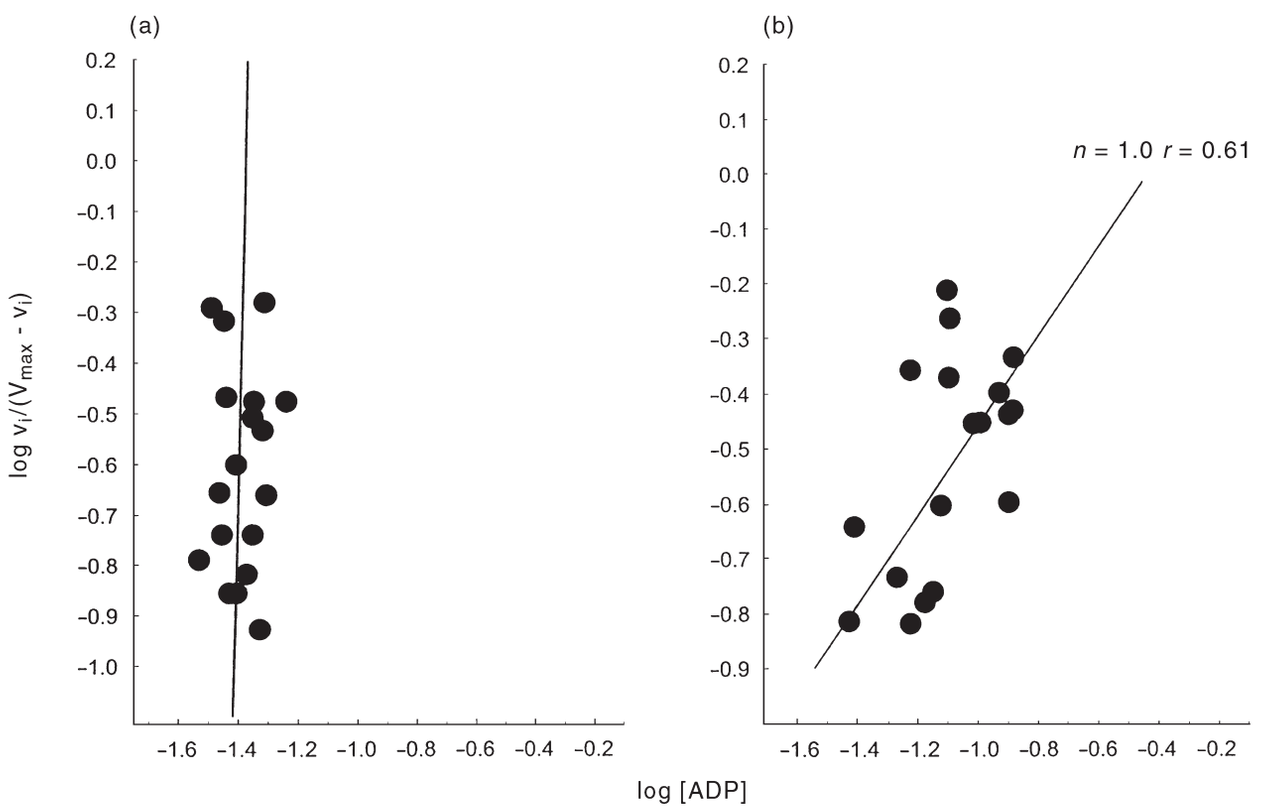

Fig. 3 Graphical analysis using the Hill equation (see text) is demonstrated for mature (a) and newborn sheep. The data for mature hearts reveal the lack of change in ADP that occurs with increases in myocardial respiration. The coefficient for newborn data (1.0) is consitent with predictions for Michaelis–Menten or first-order kinetics. v_i, myocardial oxygen consumption rate; V_{max}, maximal oxidative phosphorylation rate. Reprinted with permission of the American Physiological Society from Krueger *et al.*[32]

increases in β-F1ATPase transcript levels are not accompanied by comparable changes in that protein.[7] Thus, these proteins may be subject to post-transcriptional regulation.

THYROID HORMONE REGULATION OF ANT

Thyroid hormone specifically promotes transcriptional expression for ANT1.[26] This phenomenon could explain, in part, the elevation in ovine heart mRNA expression, which occurs after the neonatal thyroid hormone surge.[18] Lambs thyroidectomized immediately after birth demonstrate persistence of immature respiratory kinetics at age 30 days. The ADP-dependent respiratory control pattern occurs in association with reduced accumulation of ANT mRNA and protein, but with normal quantities of βF1-ATPase within the myocardium.

Conformation or efficiency at individual ANT exchange sites could also be thyroid hormone dependent. Such an influence could offer alternative mechanisms for the apparent thyroid control of respiratory maturation in the heart. For instance, Dummler *et al*.[29] demonstrated T_3 upregulation of ANT2 in normal rat hearts. Conceivably, thyroidectomy induced changes in ANT isoform distribution could alter ANT protein function in the sheep heart model. However, reductions in both ANT2 and ANT3 mRNA levels similar to those observed for ANT1 occur in the sheep heart after thyroidectomy, indicating that no relative changes in isoform and, presumably, isozyme composition occurred.[18] Although ANT protein content is reduced by thyroidectomy, ADP/ATP transport efficiency is not diminished at individual exchange sites. Thus, thyroid deficiency does not appear to alter conformation or function of this mitochondrial membrane protein. Therefore, thyroid hormone appears to regulate maturation of myocardial respiratory control through nuclear-mediated increases in ANT protein content and not through qualitative changes in functional capacity at individual exchange sites.

THE ANT AND MYOCARDIAL REMODELLING

Developmental programming for ANT and other mitochondrial proteins may be relevant to the heart, which exhibits remodelling in response to pathological processes. Myocardial infarction induces left ventricular hypertrophy and hypertrophy in the porcine heart (discussed by Zhang in this series[30]).[6,31] These pigs demonstrate either haemodynamic compensation or maladaptation expressed as clinical symptoms of congestive hearts. Maladaptive hearts demonstrate ADP-dependent respiratory kinetics similar to those observed in the maturing heart, while compensated hearts exhibit mature-type respiratory control patterns during increases in work state.[6,31] Accordingly, quantitative deficits in ANT protein, as well as in F0-F1-ATPase components are apparent in maladaptive hearts.[5] Compensated hearts demonstrate normal levels of these proteins. Coordination between protein and steady state mRNA levels occurs for both ANT and βF1-ATPase, implying that regulation of these proteins depends, in part, on changes in transcriptional rates and/or stability of transcriptional products. Furthermore, results from the porcine studies imply that reiteration of fetal or immature programming for these proteins represents a maladaptive event, which may also restrict myocardial oxidative reserve or capacity. Thus, ANT and other mitochondrial proteins participate in the pathogenesis of myocardial remodelling and heart failure.

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