The addition of glutamine as a major nutrient to cultured neonatal rat cardiomyocytes produced an increase in myocyte size and the organization of actin into myofibrillar arrays. The cellular response was associated with increased abundance of the mRNAs encoding the contractile proteins, α-myosin heavy chain and cardiac α-actin, and the metabolic enzymes, muscle carnitine palmitoyl transferase I and muscle adenylsuccinate synthetase (ADSS1). Adss1 gene expression was induced ~5-fold in glutamine-treated rat neonatal cardiac myocytes. The induction was mediated through the protein kinase A and mammalian target of rapamycin signaling pathways and required a cyclic AMP response element associated with the promoter region of the Adss1 gene. These results highlight glutamine as a major nutrient regulator of cardiac gene expression and identify protein kinase A and mammalian target of rapamycin signaling pathways as mediators of the cardiomyocyte transcriptional response.

Cardiac hypertrophy is an adaptive process accompanied with a series of changes in gene expression allowing the heart to maintain or increase cardiac output in response to increased workload (1–3). Physiological hypertrophy refers to the enlargement of the heart resulting from repeated endurance exercise. This form of cardiac hypertrophy is both beneficial and reversible. It is characterized by increased total RNA and total protein with increased abundance of the adult contractile isoforms, α-myosin heavy chain (α-MHC) and cardiac α-actin. The adult isoforms of the contractile proteins provide greater contractility making it possible to pump more blood per contraction than the fetal isoforms (4, 5). These features make the physiological hypertrophic heart more efficient and powerful. Physiological hypertrophy also occurs during the neonatal period as the heart adapts to the increased energy demands of postnatal life (6) and during pregnancy in response to increased maternal cardiac demand (7). Prolonged hypertrophy, usually secondary to other pathology (e.g. hypertension), often leads to irreversible cardiomyopathy and heart failure. The pathological hypertrophic response is characterized by the transcriptional activation of fetal genes encoding contractile proteins such as β-MHC and skeletal α-actin (8, 9). The synthesis of atrial natriuretic factor (ANF) is also induced, presumably mimicking the cardiac response to pressure overload (10). These changes are believed to reflect a change in gene expression intended to achieve an “energy sparing” rather than a “contractile efficiency” status and illustrate the fact that cardiac gene expression is closely linked to energy demand (11). The intracellular signaling pathways that mediate the cardiac hypertrophic response are poorly understood as are the molecular mechanisms controlling whether a hypertrophic response is pathological or physiological.

In recent years, efforts to identify signaling pathways associated with cardiac hypertrophy have largely focused on the role of calcium signaling in mediating the cardiac hypertrophic response (12). In this regard the calcineurin-NFAT signaling pathway has received the most attention (12, 13). However, in eukaryotic cells one of the most important intracellular transducers of growth-related signaling is the rapamycin-sensitive pathway associated with mammalian target of rapamycin (mTOR), a phosphatidylinositol kinase-related protein kinase that is regulated in response to growth factors and nutritional status (14). mTOR functions at a nexus of protein kinase signaling, receiving input from numerous upstream signaling pathways and delivering instructions to a variety of downstream effectors controlling the cellular response to mitogenic stimuli (15). mTOR plays an especially important role in sensing and responding to nutrient status, particularly amino acid availability (16). Downstream consequences of mTOR activation include effects on transcription, translation, protein degradation, and cytoskeletal organization (16). Very little is known regarding the role of mTOR-mediated signaling in cardiac hypertrophy and cardiac gene regulation. We chose to use amino acid availability, specifically that of glutamine, as an experimental tool to probe the role of mTOR signaling in regulating cardiac gene expression.

Glutamine is the most abundant amino acid in the blood, with a concentration of ~2.5 mM (17, 18). Recent studies indicate that glutamine is a major gluconeogenic precursor and vehicle for interorgan carbon transport in man (19). It has long been known that under certain physiological circumstances glutamine serves as major fuel for the gut (20, 21), the kidney (22), and the immune system (23–26). Many lines of cultured mammalian cells utilize glutamine, in preference to glucose, as...
PKA and mTOR Mediate Cardiac Hypertrophic Gene Regulation

their major carbon source to meet energetic and biosynthetic needs (24, 25). Available information suggests that glutamine serves as a significant source of energy for the heart (27–30). Glutamine perfusion of the ischemic heart provides significant protection from damage resulting from loss of energy charge (31–33). We show here that the addition of glutamine as a major nutrient for cultured cardiomyocytes produces an increase in myocyte size and in the organization of actin into myofibrillar arrays. The maturation of cardiomyocytes is associated with the in vivo H-89, a protein kinase A inhibitor, and ANF, are not induced. However, the adult isoforms of two metabolic enzymes, muscle carnitine palmitoyl transferase I (CPT-1) and muscle adenylosuccinate synthetase (ADSS1), were also induced. These are the muscle-specific isoforms of enzymes associated with cardiac fatty acid metabolism and adenine nucleotide metabolism, respectively. Adss1 gene expression is induced —5-fold in glutamine-treated rat neonatal cardiac myocytes. The induction is mediated through the protein kinase A (PKA) and mTOR signaling pathways and requires a cyclic AMP response element (CRE) associated with the promoter region of the Adss1 gene. These results highlight glutamine as a major nutrient regulator of cardiac gene expression and show that PKA and mTOR signaling pathways are required for the cardiomyocyte transcriptional response.

MATERIALS AND METHODS

Rat Neonatal Cardiac Myocyte Culture and Glutamine Treatment—Neonatal rat cardiac myocytes (1–2 days old) were isolated as described previously (13) and grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% calf serum (HyClone). Cells were plated in six-well dishes (21 cm² per well) at a density of 5 × 10⁵ cells per well for RNA isolation, total cellular protein isolation, and transient transfection experiments (34). For some experiments cells were plated on coverslips for fluorescent microscopy. After 24 h cells were rinsed and then maintained for the remainder of the experiment in serum-free DMEM supplemented with 1% bovine serum albumin. The cardiac myocytes on the coverslips were treated by glutamine from 4 to 16 mM in serum-free DMEM for 24 h. All the cardiac myocytes used for RNA isolation, total protein isolation, and transient transfections were treated with 16 mM glutamine. Other cardiac myocytes were maintained in the serum-free DMEM without glutamine as control. For experiments with 1 μM H-89, a protein kinase A inhibitor, or 5 μM G06983, a protein kinase C inhibitor, was added to both the control and glutamine-treated cells. Forskolin was added to both the control and glutamine-treated cells at 1 μM concentration.

Fluorescent Microscopic Techniques—The morphological changes in cardiac myocyte size and sarcomere alignment were visually determined for control and glutamine-treated cells. After 48 h on coverslips the cells were permeabilized with 0.5% Triton X-100, fixed in 3.7% formaldehyde (Sigma), and stained for actin with BODIPY FL phallacidin (Molecular Probes, Inc., Eugene, OR). The fluorescent images were viewed with an Olympus BX60 fluorescence microscope equipped with dark-field optics and photographed using a SPOT digital camera (Diagnosticom, Sterling Height, MI).

Total Protein Isolation—Following the pretreatment by different inhibitors for 30 min and then with glutamine or angiotensin II (Ang II) for another 30 min, cardiomyocytes were extracted with lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in 50 mM NaCl, 20 mM Tris-HCl, pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml of aprotinin, 10 μg/ml leupeptin, 5 mM benzamidine, 1 mM EDTA, 5 mM N-ethylmaleimide, 50 mM NaF, 1 mM sodium orthovanadate, 25 mM glycerophosphate, and 100 mM ascorbic acid. The cell lysate was centrifuged at 14,000 rpm for 30 min, and the protein concentration in the supernatant was determined using BCA (Pierce) reagent (34).

Western Blot Analysis to Detect S6K1 and TSC2—Total cellular protein extracts (10–15 μg) were subjected to SDS-PAGE, transferred to nitrocellulose membranes at 100 volts for 1 h. The blot was blocked with 5% milk in TBST solution for 1 h at room temperature and then incubated with polyclonal rabbit anti-p70S6 kinase (i.e., S6K1) and anti-phosphothreonine 389-p70S6 kinase, anti-tuberin, and anti-phosphothreonine-1462-tuberin (Cell Signaling Technology, Inc., Beverly, MA) at 1:5000 dilution in 1% milk-TBST overnight at 4 °C, respectively, and followed 5-min washes in TBST three times. Finally the blot was incubated for 1 h with anti-rabbit IgG (Dako) for 20000 dilution in 1% milk-TBST overnight at 4 °C, and followed with 5-min washes in TBST three times. The signal was detected by the ECL detection system (Amer sham Biosciences).

RNA Isolation and Northern Hybridization—Total RNA was isolated by using a Trizol RNA isolation kit (Invitrogen). Total cellular RNA (10 μg) was subjected to electrophoresis on 1% agarose formaldehyde gels and blotted onto GeneScreen Plus membranes (35). The membranes were hybridized with the 32P-labeled probes as described previously (35), and then washed away with denaturing solutions as described previously (35), and transferred to nylon membranes. RNA was cross-linked to the membrane by ultraviolet irradiation and incubated with an Adss1 probe as described before (13). Following multiple stringent washings the membranes were subjected to autoradiography for 24–48 h at ~70 °C.

Preparation of Plasmids and Transfection—The reporter construct, 1.9Adss1/CAT, has been described before (13). The putative CAMP response element-binding protein binding site within 1.9Adss1/CAT was disrupted using a PCR site-directed mutagenesis kit from Stratagene (13). The consensus CRE, TGAACGCTA, was mutated to TGgtGTCA as published previously (35). Rat neonatal cardiac myocytes were cultured as above, transfected with plasmid vectors, and assayed for CAT activity and β-galactosidase as described (13, 36).

Quantitative Reverse Transcriptase-PCR Analysis—Specific quantitative assays were developed for rat α-MHC, β-MHC, skeletal α-actin, cardiac α-actin, c-fos, ANF, muscle CPT-1, and cyclophilin, respectively, from published sequences (37–39). In each case 100 ng of total RNA (extracted as described above) was reverse transcribed for 30 min at 42 °C with 400 ng specific reverse primer, 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris buffer, pH 8.3 (at 20 °C), 500 μM deoxynucleotides and 0.1 μM reverse transcriptase (Superscript II; Invitrogen) in a total volume of 20 μl. Subsequently, 8 μl of the reverse transcriptase reaction was used for quantitative two-step PCR (95 °C for 1 min, followed by 40 cycles of 95 °C for 12 s, 60 °C for 1 min) in the presence of reverse primer, 100 μM specific fluorogenic probe, 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris buffer, pH 8.3 (at 20 °C), 200 μM deoxynucleotides, and 1.2 units of Taq polymerase (Sigma) in a final volume of 50 μl.

Statistical Analysis—All values are expressed as mean ± S.E. Data were analyzed for statistical significance using GraphPad Prism software. Statistical significance was determined by Student’s t test or analysis of variance test. A value of p < 0.05 was interpreted to mean that observed experimental differences were statistically significant.

RESULTS

Glutamine Induces Physiological Cell Growth and Maturation—To study the role of glutamine in the growth and differentiation of cardiac myocytes, cells were incubated in serum-free minimal medium in the presence of different concentrations of glutamine (4–16 mM). After 24 h the cells were fixed and subsequently stained with the immunofluorescent probe, BODIPY phallacidin, to identify actin filaments. Dramatic differences between the control and glutamine-treated cardiac myocytes were evident, not only in size but also in the organization of actin from punctate cores of actin characteristic of immature cardiac myocytes into striated arrays of well-organized mature actin filaments. (Fig. 1). These alterations were accompanied by a significant increase in protein content (5.4 ± 0.25 to 11.36 ± 1.13 mg/cm² cells, p < 0.05) and RNA content (1.85 ± 0.85 to 3.32 μg/mg cell, p < 0.05) in the glutamine-treated cells. These results indicate that the addition of glutamine to minimum essential medium leads to an increase in cardiomyocyte size (hypertrophy) and the organization of actin into myofibrillar arrays (maturation). Similar results were obtained with certain other amino acids, including alanine, aspartate, asparagine, and glutamate (data not shown). Because glutamine is the most abundant amino acid in the circulation and because of the special importance of glutamine in energy metabolism and nitrogen metabolism we chose to focus on glutamine for the remainder of our studies.
Fig. 1. Glutamine induces growth and maturation of rat neonatal cardiac myocytes. Rat neonatal cardiomyocytes were cultured in the serum-free medium without and with glutamine (4–16 mM). After 24 h, cardiomyocytes were fixed and stained with BODIPY FL phallacidin to visualize actin. The original photos were taken under ×400 magnification. Bars, 500 μm.

To evaluate the impact of glutamine on the pattern of cardiomyocyte gene expression we used quantitative PCR to determine the abundance of specific mRNAs encoding a diverse group of proteins associated with cardiac hypertrophy. We initially examined mRNAs encoding various cardiac contractile proteins, a metabolic enzyme, a peptide hormone, and a transcription factor. The results (Fig. 2) show that the abundance of cardiac α-actin and α-MHC mRNAs increased in response to the presence of glutamine. Messenger RNA encoding CPT-1, a mitochondrial transport protein associated with fatty acid metabolism, was also induced by the presence of glutamine. The abundance of mRNAs encoding skeletal α-actin and β-MHC, fetal isoforms characteristic of hypertrophic cardiomyopathy, did not change significantly following glutamine treatment. Evaluation of mRNA from control and glutamine-treated cells also showed that the cardiac hypertrophic marker gene encoding the polypeptide hormone, ANF, was not induced. The abundance of c-fos mRNA increased slightly in response to the presence of glutamine. Overall, these results show that stimulation of cardiomyocyte growth and maturation by glutamine was accompanied by increased expression of genes encoding the adult isoforms of cardiac proteins (e.g. cardiac α-actin, α-MHC, and muscle CPT-1), with no effect on the fetal isoforms. Genes normally induced as a result of pathological hypertrophy, i.e. those encoding skeletal α-actin, β-MHC, and ANF, were not induced in response to the presence of glutamine as a major nutrient.

Glutamine Stimulates Increased Transcriptional Activity of the Adss1 Gene—ADSS1 is an enzyme of purine nucleotide synthesis that functions at a metabolic branch point controlling the synthesis of adenine nucleotides. Glutamine is a required biosynthetic precursor and intermediate in adenine nucleotide synthesis. Additionally, Adss1 gene expression in cardiomyocytes is stimulated in response to cardiac hypertrophic stimuli (13, 40). For these reasons we chose Adss1 as a marker gene to study the cardiac transcriptional response to glutamine. We initially determined the effect of glutamine on the abundance of Adss1 mRNA in cultured rat neonatal cardiomyocytes. Northern hybridization showed that the abundance of Adss1 mRNA was elevated relative to control cardiomyocytes by 6 h of glutamine exposure and continued to increase for at least 24 h (Fig. 3A). To determine whether the increased abundance of Adss1 mRNA reflected increased transcription of the Adss1 gene we conducted transfection experiments with Adss1/CAT reporter constructs. Transfection assays showed that Adss1 promoter activity increased 6–7-fold in response to glutamine (Fig. 3B). These results imply that intracellular signaling pathways link the presence of glutamine with transcriptional activation of the Adss1 gene.

Activation of the Adss1 Promoter by Glutamine Is Mediated through the Protein Kinase A Pathway—Previous studies indicate that glutamine controls gene expression through cyclic AMP-mediated signaling pathways in epithelial cells (21). In an effort to identify signaling pathways associated with the glutamine induction of Adss1 gene expression in cardiomyocytes, we tested the effects of forskolin (an activator of adenylyl cyclase) and glutamine on Adss1 promoter activity. In addition, we tested the effect of protein kinase A and protein kinase C inhibitors on Adss1 promoter activity in the presence and absence of glutamine. Transfection assays showed that forskolin treatment alone increased Adss1 transcriptional activity ~4-fold. Glutamine alone resulted in a 6-fold induction. Glutamine and forskolin together increased Adss1 promoter activity in an additive way for a total of 11-fold. The protein kinase C inhibitor (G06983) had no effect on Adss1 promoter activity, suggesting that protein kinase C is not involved in the activation of the Adss1 promoter by glutamine (data not shown). In contrast, the protein kinase A inhibitor, H-89, completely blocked the activation of the Adss1 promoter by glutamine but had no effect on its basal expression in the absence of glutamine (Fig. 4).
These results suggest that the protein kinase A signaling pathway contributes to the induction of Adss1 promoter activity by glutamine. However, although H-89 is well known as a PKA inhibitor, it is also a potent inhibitor of p70S6K1, a growth-regulated kinase under the control of mTOR. Thus, it is possible that the mTOR signaling pathway is involved in the activation of Adss1 gene expression by glutamine.

Activation of the Adss1 Promoter by Glutamine Is Mediated in Part by the mTOR Signaling Pathway—Studies in yeast (41) and Xenopus laevis (42) have highlighted the importance of mTOR in the cellular response to glutamine availability. Recent studies have linked the mTOR pathway with cAMP-induced transcriptional events suggesting an interplay between mTOR and cAMP nutrient signaling pathways (43–45). To explore the role of mTOR in the regulation of Adss1 gene expression we determined the effect of rapamycin, an inhibitor of mTOR activity, on the induction of Adss1 promoter activity following glutamine treatment. Transfection assays showed that glutamine treatment resulted in a 4-fold induction of Adss1 promoter activity and that this induction was inhibited by ~/50% by the presence of rapamycin (Fig. 5). Similar results were obtained with angiotensin II in the absence and presence of rapamycin (Fig. 5). These results suggest that mTOR activation contributes to the induction of Adss1 gene expression in response to glutamine, a nutrient, and angiotensin II, a growth factor.

A key downstream mediator of mTOR activation is the protein kinase, p70S6K1 (46–48). Activation of p70S6K1 is characterized by the phosphorylation of a specific threonine residue at amino acid position 389. The phosphorylation of p70S6K1 at threonine 389 is a feature commonly used to monitor the activation status of mTOR (48). We, too, used this approach to

Fig. 3. Glutamine-stimulated increased Adss1 mRNA abundance and increased transcription of the Adss1 gene. A, total RNA was isolated from rat neonatal cardiomyocytes treated without and with glutamine for up to 48 h. Adss1 mRNA abundance was determined by Northern blot hybridization using an Adss1 cDNA probe. The results are representative of three independent experiments. B, 1.9 Adss1/CAT was co-transfected with β-galactosidase plasmid into rat neonatal cardiomyocytes without (−) and with (+) glutamine. The expression of 1.9Adss1/CAT construct was expressed as a fold induction over that without glutamine (−). The results are expressed as mean ± S.E. of three independent determinations. *, p < 0.05.

Fig. 4. Transactivation of the Adss1 promoter by glutamine is through the protein kinase A pathway. 1.9 Adss1/CAT was co-transfected with a β-galactosidase construct into cardiomyocytes without (−) and with (+) glutamine treatment. Cultures were maintained in the absence or presence of Forskolin (Fsk) or H-89 (protein kinase A inhibitor). The expression of the 1.9 Adss1/CAT construct was expressed as a fold induction over that observed from cultures lacking the glutamine treatment. The results are expressed as mean ± S.E. of three independent determinations. *, p < 0.05 versus control without glutamine treatment and **, p < 0.05 versus glutamine treatment.
determine the effect of glutamine treatment on mTOR activity. Treatment of serum-starved cardiomyocytes with angiotensin II or glutamine resulted in the phosphorylation of p70S6K1 at Thr-389 (Fig. 6A). In each case the increased phosphorylation of p70S6K1 was blocked by the presence of rapamycin (Fig. 6A). These results provide confirmation that the mTOR signaling pathway is activated in response to treatment with Ang II or glutamine. The PI3 kinase inhibitor, LY294002, blocked the phosphorylation of p70S6K1 at Thr-389 induced by Ang II but not by glutamine. These results indicate that Ang II activates mTOR through PI3 kinase signaling pathway, whereas glutamine does not.

To further assess the role of mTOR signaling in regulating Adss1 gene expression we assessed the phosphorylation status of tuberin, an upstream regulator of mTOR activity (14). Tuberin serves as a negative regulator of mTOR activity, a feature that is prevented when tuberin is phosphorylated at Thr-1462 (49). Thus activation of mTOR is expected to be associated with the phosphorylation of tuberin. The impact of Ang II or glutamine treatment on the phosphorylation of tuberin was determined using a phosphopeptide-specific antibody that specifically recognized phosphorylation at Thr-1462. The results presented in Fig. 6B show enhanced phosphorylation of tuberin in response to Ang II or glutamine treatment. These findings provide additional evidence that Ang II or glutamine treatment of serum-starved neonatal cardiomyocytes results in the activation of the mTOR signaling pathway.

A CRE in the 5' Flanking Region of the Adss1 Gene Is Essential for Induction by Glutamine—The research presented above indicates that glutamine activates Adss1 gene expression through signaling pathways involving increased intracellular cyclic AMP and the activation of protein kinases, PKA, mTOR, and p70S6K1. PKA and p70S6K1 act upstream of transcription factors that activate gene expression through the use of CREs associated with target genes (44, 45). Consistent with the potential involvement of these pathways we identified a CRE in the promoter region of the Adss1 gene. To determine whether this site is critical for the glutamine-mediated transcriptional induction of the Adss1 gene an Adss1 reporter construct with a mutationally destroyed CRE site was prepared. Transfection assays showed that site-directed mutation of CRE in the Adss1 promoter region completely abolished the activation of the Adss1 promoter by glutamine (Fig. 7). Mutation of the CRE site did not lower basal expression of the Adss1 reporter construct. These results indicate that the CRE associated with the Adss1 promoter region is required for activation by glutamine.

**DISCUSSION**

Considerable attention has been devoted to the study of glucose and fatty acids as metabolic fuels for the heart (50–52). Very little attention has been given to the role of amino acids as cardiac fuels and their impact on cardiac energy metabolism and gene expression. Here we show that glutamine, the most abundant amino acid in blood, can induce cardiomyocyte growth and maturation accompanied with increased abundance of mRNAs encoding the adult isoforms of contractile proteins (α-MHC and cardiac α-actin) and metabolic enzymes (CPT-1 and ADSS1). The Adss1 gene was chosen for further analysis, because considerable information is available regarding the regulation of this gene in the heart (36, 53). Our results revealed the importance of the protein kinase A and mTOR signaling pathways in the glutamine-mediated induction of Adss1 gene expression. A cyclic AMP response element located in the promoter region is required for glutamine-induced Adss1 gene activation and likely functions downstream of PKA and mTOR signaling pathways. The results reported here identify...
glutamine as a major nutrient regulator of cardiac gene expression and show that PKA and mTOR signaling pathways are associated with the cardiomyocyte transcriptional response.

For much of the research reported here we used the Adss1 gene as a reporter to monitor the cardiomyocyte transcriptional response to the presence of glutamine. ADSS1 functions at a critical branch point in purine nucleotide metabolism where it controls the synthesis of adenine nucleotides (AMP, ADP, and ATP) from the purine nucleotide intermediate IMP (53). Glutamine is an essential source of nitrogen in two reactions leading to the synthesis of adenine nucleotides, providing two of the four nitrogens making up the purine ring. Because of the importance of ADSS1 in cardiac adenine nucleotide metabolism and the essential role of glutamine in adenine nucleotide synthesis we chose the Adss1 gene for more in depth analysis of the effects of glutamine on cardiac gene expression. The Adss1 gene is well suited for our studies, because considerable information is available regarding transcription factors regulating Adss1 gene expression in the heart (13, 36, 53). The Adss1 gene is activated early in the development of the cardiac lineage and is massively up-regulated during the neonatal period to achieve very high levels of expression in the postnatal heart (36, 53). Developmental control of Adss1 gene expression is achieved through the use of a cardiac-specific enhancer that confers proper developmental activation and neonatal enhancement in the cardiac lineage (36, 53). The cardiac enhancer region contains essential binding sites for numerous well known cardiac transcription factors including NKX2.5, GATA4, MEF2C, E12, HAND1, and HAND2 (36, 53).

Adss1 gene expression is also induced by a variety of hypertrophic stimuli including electrical stimulation (13), angiotensin II (40), and aortic banding (40). In these cases Adss1 gene activation is mediated through the action of NFAT transcription factors acting downstream of calcineurin and calmodulin. Adss1 reporter constructs have proven to be sensitive and reliable markers of cardiac gene expression in cardiomyocyte transfection and transgenic mouse experiments (36). Our results suggest that the Adss1 gene should serve as an excellent reporter to assess the mechanisms by which PKA and mTOR signaling pathways regulate the cardiac transcriptional response to glutamine.

mTOR is an evolutionarily conserved nutrient sensor that directs the cellular response to nutrient status (15, 16, 48),

FIG. 7. A cyclic AMP response element in the 5’ flanking region of the Adss1 gene is essential for induction by glutamine. Either a wild-type 1.9Adss1/CAT construct or one with a mutant CRE was co-transfected with a β-galactosidase construct into the cardiomyocytes in the presence or absence of glutamine treatment. The transcriptional activity of the wild-type and CRE mutant 1.9Adss1/CAT constructs was expressed as fold inductions over that obtained from cells lacking glutamine treatment. The mutation of CRE site in Adss1 promoter significantly inhibits the activation of reporter gene in response to glutamine. The results are expressed as mean ± S.E. of three independent determinations. *, p < 0.05 versus control without glutamine treatment and **, p < 0.05 versus glutamine treatment.

FIG. 8. Signaling pathways mediating the cardiac transcriptional response to glutamine and Ang II. We have shown here that mTOR signaling is a common feature of both glutamine-induced and Ang II-induced Adss1 gene activation in neonatal cardiomyocytes. According to our hypothesis, glutamine activates mTOR via PKA signaling, whereas Ang II activates mTOR through PI3 kinase signaling. Glutamine- and Ang II-induced signaling converge on Akt/PKB, an upstream regulator of mTOR. The Adss1 transcriptional response to PKA and mTOR signaling relies on a cyclic AMP response element associated with the gene. In previous studies we have shown that Ang II-mediated activation of Adss1 gene expression requires calcineurin/NFAT signaling (13). The Adss1 transcriptional response to Ang II requires an NFAT binding site. The results presented here highlight the fact that the cardiac transcriptional response to nutrient and growth factor signaling relies on the mTOR signaling pathway. See text for more details. CREM, CRE-binding protein modulator; PDK, PI3 kinase-dependent kinase.
especialiy the availability of amino acids. Recent studies have also identified tuberin (the product of the TSC2 gene) as a nutrient sensor (14, 49). In a complex with hamartin (product of the TSC1 gene), tuberin functions as a negative regulator of mTOR, a role that is prevented by phosphorylation of tuberin at position Thr-1462 (14). Thus, it is reasonable to assume that calcium signaling is sufficient to induce myocyte hypertrophy (12). Forced activation of calcium-sensitive signaling pathways activated in cardiac myocytes in response to hypertrophic stimuli provides a clue regarding the mechanism by which mTOR and tuberin function as nutrient sensors. We have shown here that the presence of glutamine, as the major nutrient, activates the cAMP signaling pathway. Thus, our hypothesis provides a clue regarding the mechanism by which mTOR and tuberin sense nutrient status and regulate the expression of a variety of signaling pathways (e.g. phosphorylation of p70S6K1 at threonine 389), leading to cell growth. In the context of this hypothesis it is noteworthy that other nutrients such as glucose (58, 59) and glucosamine also activate mTOR signaling but appears to do so through the mTOR pathway in mediating the Ang II hypertrophic response. Our results indicate that the Adss1 gene expression is sensitive to inhibition by cyclosporin A (an inhibitor of calcineurin) and requires an intact NFAT binding site in the promoter region of the Adss1 gene. The results presented here highlight the fact that, like glutamine, Ang II also activates the mTOR signaling pathway in mediating the Ang II hypertrophic response. Our results indicate that the Adss1 gene will serve as an excellent reporter to identify components of the mTOR signaling pathways required for the cardiac transcripational response to the hypertrophic effects of nutrients and growth factors.

Acknowledgment—We are grateful to Dr. Stanislaw Stepkowski for supplying us with rapamycin.

REFERENCES

1. Olson, E. N., and Molkentin, J. D. (1999) Circ. Res. 84, 623–632
2. Sussman, M. A., Lim, H. W., Gude, N., Taigen, T., Olson, E. N., Robbins, J., Colbert, M. C., Guallberto, A., Wieczorek, D. F., and Molkentin, J. D. (1998) Science 281, 1690–1693
3. Sugden, P. H. (1999) Circ. Res. 84, 633–646
4. Kozakova, M., Galetta, F., Gregorini, L., Bigalli, G., Franzoni, F., Giusti, C., and Palombo, C. (2000) Hypertension 36, 343–349
5. Li, Z. B., Gao, Y. Q., and Tang, Z. S. (1998) Sheng Li Xue Bao 50, 551–556
6. Shiojima, I., Yefremavishvili, M., Luo, Z., Kureishi, Y., Takahashi, A., Tao, J., Rosensweig, A., Kahn, C. R., Abel, E. D., and Walsh, K. (2002) J. Biol. Chem. 277, 37670–37677
7. Mone, S. M., Sanders, S. P., and Colan, S. D. (1996) Circulation 94, 667–672
8. Izumo, S., Lompre, A. M., Matsukura, R., Koren, G., Schwartz, K., Nadal-Ginard, B., and Mahdavi, V. (1987) J. Clin. Invest. 79, 970–977
9. Schwartz, K., de la Bastide, D., Bouvetier, P., Olivier, P., Alonso, S., and Buckingham, M. (1986) Circ. Res. 59, 551–555
10. Anghese, H., Nakan, S., Saito, Y., Morii, N., Sugawara, A., Yamada, T., Itoh, H., Shiono, S., Mukoyama, M., and Ohkubo, H., et al. (1988) Circ. Res. 62, 926–930
11. Schwartz, K., Lecarpentier, Y., Martin, J. L., Lompre, A. M., Mercadier, J. J., and Swynghedauw, B. (1981) J. Mol. Cell. Cardiol. 13, 1071–1075
12. Molkentin, J. D., Lu, R. J., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R., and Olson, E. N. (1998) Cell 93, 215–228
13. Xia, Y., McMillin, J. B., Lewis, A., Moore, M., Zhu, W. G., Williams, R. S., and Kellems, R. E. (2000) J. Biol. Chem. 275, 1855–1863
14. Gao, X., Zhang, Y., Arrazola, P., Hino, O., Kobayashi, T., Yeung, R. S., Ru, B., and Pan, D. (2002) Nat. Cell Biol. 4, 699–704
15. Schmelze, T. and Hall, M. N. (2000) Cell 103, 253–262
16. Rohde, J., Heitman, J., and Cardenas, M. E. (2001) J. Biol. Chem. 276, 9583–9586
17. Abumrad, N. N., Rabin, D., Diamond, M. P., and Lacy, W. W. (1981) Metabolism 30, 936–940
18. Ham, R. G., and McKenna, W. L. (1979) Methods Enzymol. 58, 44–93
19. Narhian, N., Bucci, A., Perrelli, G., Stumvoll, M., Dailey, G., Bier, D. M., Toft, I., Jensen, T. G., and Gerich, J. E. (1995) J. Clin. Invest. 95, 272–277
20. Rhoads, J. M., Argenzio, R. A., Chen, W., Rippe, R. A., Westwick, J. K., Cox, A. D., Berschneider, H. M., and Brenner, D. A. (1997) Am. J. Physiol. 273, G943–G953
21. Rhoads, J. M., Argenzio, R. A., Chen, W., Graves, L. M., Licato, L. L., Bilkisager, A. T., Smith, J., Gatzky, J., and Brenner, D. A. (2000) Gastroenterology 118, 90–109
22. Kunz, W. S., Kriehue, L., and Gellerich, F. N. (1992) Biochim. Biophys. Acta 1109, 329–331
23. Wu, G. Y., Field, C. J., and Marliess, E. B. (1991) Biochim. Biophys. Acta 1115, 166–173
24. Wu, G. Y., Field, C. J., and Marliess, E. B. (1991) Biochim. Biophys. Acta 1080, 801–808
25. Wu, G. Y., Field, C. J., and Marliess, E. B. (1991) Biochim. Biophys. Acta 1080, 441, 299–305
26. Brand, K. (1985) Biochem. J. 228, 353–361
27. Nelson, D., Rumsey, W. L., and Erecinska, M. (1994) Arch. Biochem. Biophys. 314, 376–383
28. Nelson, D., Rumsey, W. L., and Erecinska, M. (1999) Biochem. J. 328(Pt 2), 559–564
29. Rennie, M. J., Low, S. Y., Taylor, P. M., Khogali, S. R., Yao, P. C., and Ahmed, A. (1998) Adv. Exp. Med. Biol. 441, 299–305
30. Szwaja, J., Wic, B. M., and Kennell, D. E. (1983) J. Cell. Biol. 115, 320–330
31. Khogali, S. E., Harper, A. A., Lyall, J. A., and Rennie, M. J. (1996) Biochem. J. 319, 819–827
32. Rennie, M. J., Khogali, S. E., Low, S. Y., McDowell, H. E., Hundal, H. S., Ahmed, A., and Taylor, P. M. (1996) Biochem. Soc. Trans. 24, 869–873
33. Rennie, M. J., Bowell, J. L., Bruce, M., and Khogali, S. E. (2001) J. Nutr. 131, 2492–2498
34. Xia, Y., Wen, H. Y., and Kellems, R. E. (2002) J. Biol. Chem. 277, 24601–24608
35. Xia, Y., Buja, L. M., Scarpulla, R. C., and McMillin, J. B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11399–11404
36. Lewis, A. L., Xia, Y., Datta, S. K., McMillin, J., and Kellems, R. E. (1999) J. Biol. Chem. 274, 14188–14197
37. Degre, C., Shipley, G. L., Hsu, W., Han, Q., Deust, T., Moore, M. L., Stepkowski, S., Davies, P. J., and Taegtmeyer, H. (1998) Nat. Med. 4, 1269–1275
38. Degre, C., and Taegtmeyer, H. (2000) Cardiovasc. Res. 45, 538–548
39. Young, M. E., Patel, S., Ying, J., Degre, A., Ahuja, H. S., Shipley, G. L., Stepkowski, S., Davies, P. J., and Taegtmeyer, H. (2001) FASEB J. 15, 833–845
PKA and mTOR Mediate Cardiac Hypertrophic Gene Regulation

40. Wen, H. Y., Xia, Y., Young, M. E., Taegtmeyer, H., and Kellems, R. E. (2002) J. Cell. Mol. Med. 6, 235–243
41. Heitman, J., Movva, N. R., and Hall, M. N. (1991) Science 253, 905–909
42. Christie, G. R., Hajduch, E., Hundal, H. S., Proud, C. G., and Taylor, P. M. (2002) J. Biol. Chem. 277, 9952–9957
43. Fimia, G. M., and Sassone-Corsi, P. (2001) J. Cell Sci. 114, 1971–1972
44. de Groot, R. P., Ballou, L. M., and Sassone-Corsi, P. (1995) Immunobiology 193, 153–160
45. de Groot, R. P., Ballou, L. M., and Sassone-Corsi, P. (1994) Cell 79, 81–91
46. Avruch, J., Belham, C., Weng, Q., Hara, K., and Yonezawa, K. (2001) Prog. Mol. Subcell. Biol. 26, 115–154
47. Valarevic, S., and Thomas, G. (2001) Prog. Nucleic Acids Res. Mol. Biol. 65, 101–127
48. Thomas, G., and Hall, M. N. (1997) Curr. Opin. Cell Biol. 9, 782–787
49. Tee, A. R., Fingar, D. C., Manning, B. D., Kwikowski, D. J., Cantley, L. C., and Blenis, J. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 13571–13576
50. Lehman, J. J., and Kelly, D. F. (2002) Clin. Exp. Pharmacol. Physiol. 29, 339–345
51. Frey, N., and Olson, E. N. (2002) Circulation 105, 1152–1154
52. Razeghi, P., Young, M. E., Cockrill, T. C., Frazier, O. H., and Taegtmeyer, H. (2002) Circulation 106, 407–411
53. Lewis, A. L., Guicherit, O. M., Datta, S. K., Hanten, G. R., and Kellems, R. E. (1996) J. Biol. Chem. 271, 22647–22656
54. Filippa, N., Sable, C. L., Filloux, C., Hemmings, B., and Van Obberghen, E. (1999) Mol. Cell. Biol. 19, 4989–5000
55. Liu, H., and Maurice, D. H. (1999) J. Biol. Chem. 274, 10557–10565
56. Shios, T., McMullen, J. R., Kang, P. M., Douglas, P. S., Obata, T., Franke, T. F., Cantley, L. C., and Izumo, S. (2002) Mol. Cell. Biol. 22, 2799–2809
57. Manning, B. D., Tee, A. R., Logsdon, M. N., Blenis, J., and Cantley, L. C. (2002) Mol. Cell 10, 151–162
58. Schadick, K., Fourcade, H. M., Boumenot, P., Seitz, J. J., Morrell, J. L., Chang, L., Gould, K. L., Partridge, J. F., Allshire, R. C., Kitagawa, K., Haster, P., and Hoffman, C. S. (2002) Eukaryot. Cell 1, 558–567
59. Wu-Wong, J. R., Berg, C. E., and Dayton, B. D. (2002) Clin. Sci. (Lond.) 103, Suppl. 48, 418S–423S
60. Hoshijima, M., and Chien, K. R. (2002) J. Clin. Invest. 109, 849–855
Mammalian Target of Rapamycin and Protein Kinase A Signaling Mediate the Cardiac Transcriptional Response to Glutamine

Yang Xia, Hong Y. Wen, Martin E. Young, Patrick H. Guthrie, Heinrich Taegtmeyer and Rodney E. Kellemes

J. Biol. Chem. 2003, 278:13143-13150. doi: 10.1074/jbc.M208500200 originally published online January 9, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M208500200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 60 references, 29 of which can be accessed free at http://www.jbc.org/content/278/15/13143.full.html#ref-list-1