In Vitro Cell. Dev. Biol.—Animal 32:573–578, October 1996

1071-2690/96 $05.00+0.00

THE EFFECT OF ANGIOTENSIN II ON MYOSIN HEAVY CHAIN EXPRESSION IN CULTURED MYOCARDIAL CELLS

NOA SHALITIN, MENAHEM FRIEDMAN, HADASSA SCHLESINGER, YAEL BARHUM, MAURICE J. LEVY, WOLFGANG SCHAPER, AND GANIA KESSLER-ICEKSON[1]

The Basil and Gerald Felsenstein Medical Research Center, Beilinson Medical Campus, Petah-Tikva 49100, Israel (N. S., H. S., Y. B., G. K.-I.); Department of Surgery, Sackler School of Medicine, Tel-Aviv University, Israel (M. F., M. J. L.); Department of Experimental Cardiology, MPI, Bad-Nauheim, Federal Republic of Germany (W. S.)

(Received 20 September 1995; accepted 29 November 1995)

SUMMARY

Angiotensin II (AII), the principal mediator of the renin–angiotensin system, is an important regulator of vascular and cardiac homeostasis. AII has also been shown to be a regulator of cardiac hypertrophy and of the corresponding changes in amount and composition of certain tissue proteins. We examined the trophic effects of AII on cultured myocytes derived from neonatal rat ventricles and followed, by Northern blot analysis and polyacrylamide gel electrophoresis, the expression of α- and β-myosin heavy chain iso-mRNAs and isoproteins. Our findings show that a single administration of AII is sufficient to induce a trophic response in cultured beating myocytes and to enhance the expression of β-myosin heavy chain iso-mRNA and isoprotein, having no effect on α-myosin heavy chain. Induction of α-myosin heavy chain expression by thyroid hormone before AII was administered showed that AII could not potentiate a shift from α- to β-myosin heavy chain predominance. We suggest that the potency of AII to regulate the expression of myosin heavy chain isogenes is restricted to the β isoform and is overridden by thyroid hormone.

Key words: neonatal heart myocytes; angiotensin II; cellular hypertrophy; myosin heavy chains.

INTRODUCTION

Angiotensin II is a powerful vasoconstrictor and a positive inotropic and chronotropic effector of the heart. Whereas part of the regulatory effects of AII on the myocardium are secondary to its hemodynamic actions, others result from the direct interaction of AII with the cardiac cell (Baker et al., 1992). Angiotensin II has been shown to modulate myocyte contractility and to regulate its growth and gene expression. Stimulation of protein synthesis and cellular hypertrophy by AII and AII analogs was observed in cultured myocytes from embryonic chicks and from neonatal rats, the signal of AII being mediated via the AT_1 receptor subtype (Allen et al., 1988; Aceto and Baker, 1990; Baker and Aceto, 1990; Sadoshima and Izumo, 1993a). Intracardiac production of AII by a local renin–angiotensin system implicates paracrine and autocrine routes for the regulatory actions of AII on the cardiac cell (Lindpainter et al., 1988). Autocrine release of AII has been shown to mediate stretch-induced hypertrophy of cardiac myocytes in culture (Sadoshima et al., 1993; Komuro et al., 1995). Angiotensin II is mitogenic to fibroblasts of the myocardial interstitium, promoting protein synthesis and the production of extracellular matrix components (Sadoshima and Izumo, 1993a; Schorb et al., 1993; Villareal et al., 1993).

Hypertrophy of the myocardial cell, a quantitative process by definition, involves marked qualitative changes also. These include the stimulation of transcription of several classes of early response gene products which act as intracellular mediators of growth signals, and the upregulation of late response, tissue-specific proteins which alter the functional features of the heart. Part of the late response proteins are normally expressed in the embryonic heart (reviewed by Chien et al., 1993; Komuro and Yazaki, 1993). In cultured myocytes, AII-induced hypertrophy has been associated with increased amounts of rRNA and of mRNAs for the proto-oncogenes *Egr-1*, c-*fos*/c-*jun*, and c-*myc*, as well as for α-skeletal actin, atrial natriuretic peptide, endothelin, and angiotensinogen (Aceto and Baker, 1990; Baker and Aceto, 1990; Sadoshima and Izumo, 1993a, 1993b; Sadoshima et al., 1993). As yet, no effect of AII on the amount and composition of myosin heavy chains (MHCs) has been reported.

Myosin is the major component of sarcomeric thick filaments, and in the rat heart it presents two isotypes, V1 and V3, the respective homodimers of α- and βMHC. Isotype V1 generates faster speed of contraction and higher ATPase activity with lower energetic efficiency than V3 (Schwartz et al., 1981; Alpert and Mulieri, 1982). The plasticity of myocardial performance during development and under altered hormonal and physiological states has long been correlated with transitions in MHC predominance. Isotype V3 is the fetal isoform of the rodent heart and is replaced by V1 during ontogenic development (Lompre et al., 1981). Cardiac hypertrophy caused by thyroid hormone shifts the heart to αMHC predominance, whereas hypertrophy caused by pressure overload results in βMHC predominance (Mercadier et al., 1981; Izumo et al., 1987). In sus-

[1]To whom correspondence should be addressed.

tained high-renin hypertension, an increased proportion of ventricular βMHC has been attributed to the resultant hemodynamic overload and not to the AII generated by high renin activity (Buttrick et al., 1991). To find out whether the upregulation of βMHC may also be a primary response of myocytes to excess AII besides being secondary to the hemodynamic pressure, we examined myocytes grown in culture, devoid of hemodynamic load. Our findings show that a single administration of AII is sufficient to induce both hypertrophy of cultured myocytes and upregulation of βMHC iso-mRNA and isoprotein, with no marked effect on αMHC.

Materials and Methods

Cell cultures. Primary myocyte cultures were prepared as previously described (Kessler-Icekson et al., 1984; Bergman et al., 1995). In brief, ventricles from 1–2-day-old Wistar rats were dissociated enzymatically at room temperature with RDB™, a protease isolated from fig tree extract. Five to six 20-min cycles of digestion dispersed the tissue fragments completely. To reduce the proportion of nonmyocytes, we used one of two protocols. In the first protocol, the freshly dispersed cells were suspended in IB1, a "rich" serum-free medium composed of Dulbecco modified Eagle's medium (DMEM) and Ham's F12 at a 1:1 ratio, 10 m*M* HEPES at pH 7.3, 0.1% bovine serum albumin (BSA), 2 mg glucose/ml, 25 μg insulin/ml, 25 μg transferrin/ml, MEM amino acids and vitamins, 0.1 μ*M* hydrocortisone, 0.5 mg fetuin/ml, and antibiotics. The cells were seeded at a density of 1.5×10^5 per cm^2 into collagen-coated culture dishes in the presence of 0.1 m*M* bromodeoxyuridine (BrdU) (Simpson et al., 1982). Twenty-four hours later, the medium was replaced with IB2, a "poor" serum-free medium composed of DMEM : Ham's F12 (1:1), 10 m*M* HEPES at pH 7.3, 0.1% BSA, 2.5 μg insulin/ml, 2.5 μg transferrin/ml, 0.1 μ*M* hydrocortisone, and antibiotics containing BrdU. The cells were kept in this medium for two more days, then shifted to fresh BrdU-free IB2 24 h before the administration of experimental treatments.

In the second protocol to reduce the proportion of nonmyocytes, the dispersed cells were suspended in IB/FCS (DMEM : Ham's F12, 10% fetal calf serum, and antibiotics), placed in collagen-free 100-mm culture dishes, and incubated for 45–60 min. Unsettled cells were collected, BrdU was added, and the myocyte-enriched cell suspension was seeded into collagen-coated culture dishes at 1.5×10^5 per cm^2. Twenty-four hours later, the medium was replaced with BrdU-free IB2. The cells were maintained in this medium for 2–3 d until the experiments began. Myocytes of the two plating procedures had similar morphology and resumed spontaneous beating 24 h postplating, but cells from procedure 2 were beating more vigorously. We attribute this to a shorter exposure of the cultures to BrdU (24 instead of 72 h). We used either 35- and 60-mm culture dishes or 12- and 24-well culture plates, depending on the design of the experiments. Experimental treatments were given in IB2.

Determination of protein and DNA and phenylalanine incorporation. Cell cultures were rinsed three times with cold PBS-1 m*M* ethylenediaminetetraacetic acid (EDTA). The cells were scraped from the dish, collected in PBS-1 m*M* EDTA, homogenized by mild sonication, and sampled for the determination of protein and DNA. Protein was measured spectrophotometrically with BSA as a standard (Lowry et al., 1951). DNA was measured fluorimetrically with calf thymus DNA as a standard (Labarca and Paigen, 1980).

We added [^{14}C]phenylalanine (>450 mCi/mmole) to the experimental medium at 0.1–0.2 μCi/ml for 24 h. At the end of incubation, the medium was aspirated, the cultures were rinsed three times with cold PBS-1 m*M* EDTA, and overlaid with ice-cold 5% trichloroacetic acid (TCA). After 45 min in the cold, the cultures were rinsed four times with cold 5% TCA and thoroughly aspirated. Each culture received 0.45 ml of 1 m*M* NaOH-0.1% sodium dodecyl sulfate (SDS), was incubated 10 min at 37° C, and was sampled in duplicate for liquid scintillation counting.

Analysis of MHC. Isolation of myosin from the cell cultures and the electrophoretic separation of MHCs was as we previously described (Kessler-Icekson, 1988). Radiolabeled myosin was isolated from cultures incubated with [^{14}C]phenylalanine (2 μCi/ml) for 48 h. After electrophoresis, the gels were dried and exposed to Kodak film type X-OMAT-AR.

Analysis of RNA. Total cellular RNA was isolated by acid guanidinium thiocyanate phenol/chloroform extraction (Chumczynski and Sacchi, 1987). RNA at 15 or 20 μg/lane was size-fractionated on 1% agarose–formaldehyde gels, transferred to nitrocellulose membranes (Hybond-C extra) and UV- or heat-immobilized. The membranes were probed with ^{32}P-labeled oligonucleotides with T4 polynucleotide kinase and [γ-^{32}P]ATP. Forty-two nucleotide oligoprobes, 3′GGACAGGTCGTCTTTCTCGGAGCGGCAACGGTAGGGTG TTAT5 ′ for αMHC, and 3′GAGATGGGTTGGGATTCCTACGGACACTTCG GGACTCTGGAC5 ′ for βMHC, corresponded to nucleotides 1285–1327 at the nontranslated 3′ end of the respective mRNAs (Mahdavi et al., 1982). Between hybridizations with the two MHC probes, the membranes were dehybridized and exposed to X-ray film to ensure removal of previous radioactivity. Hybridizations were performed overnight at 42° C in 6XSSC, 50 m*M* phosphate buffer at pH 6.5, 10X Denhardt's solution, 1% SDS, 50% formamide, and 200 μg/ml salmon sperm DNA. The membranes were rinsed with 2XSSC-0.1% SDS four times briefly at room temperature (RT), 30 min at 65° C, 5 min at RT, and then briefly in 2XSSC at RT. The membranes were exposed to Kodak film type X-OMAT-AR, and the intensity of labeled bands was quantified by soft laser scanning densitometry (Biomed Instruments, Fullerton, CA, USA). Variations between lanes were corrected by normalization to 18S rRNA measured by hybridization with an oligoprobe specific for 18S rat rRNA (Mendez et al., 1987; Carrier et al., 1992).

Data analysis. The values obtained for replicate dishes (three or more) in an experiment were calculated relative to the mean value of the controls in the same experiment. The calculated values (% control) of identical treatments from several experiments (3–11 repetitions) were pooled and tested statistically with Student's *t*-test. Probability values lower than 0.05 were considered statistically significant.

Materials. Cell culture media and supplements were purchased from Biological-Industries, Beit Haemek, Israel. Cell culture dishes and multiwell plates were from Sterilin (Stone, England) or Nunc (Roskilde, Denmark). RDB™ was from Israel Institute for Biological Research, Ness-Ziona, Israel. Losartan was a gift of DuPont-Merck, Wilmington, DE, USA. The [^{14}C]phenylalanine and Hybond-C membranes were from Amersham, Little Chalfont, U.K. The [γ-^{32}P]ATP was from Amersham or Rotem Industries, Beer-Sheva, Israel. The T4 polynucleotide kinase was from New England Biolabs, Beverly, MA, USA, and AII and all other chemicals were from Sigma Chemical Co., St. Louis, MO, USA.

Results

AII Effect on Protein Accumulation

An increase in the amount of incorporated phenylalanine or in the protein-to-DNA ratio indicates protein accumulation and is considered a measure of myocyte hypertrophy (Simpson, 1985; Meidell et al., 1986). The maximal increase in phenylalanine incorporated into myocytes treated for 24 h with increasing concentrations of AII, each administered as a single dose, was 35%, obtained with 100 n*M* AII (Fig. 1). The trophic effect of AII was totally blocked by losartan, a nonpeptide inhibitor of the AT_1 receptor (Timmermans et al., 1991). Myocytes treated with 10 n*M* AII-100 n*M* losartan or 100 n*M* AII-1000 n*M* losartan incorporated phenylalanine at 97 ± 6.3% and 93 ± 13% of the control, respectively. A 72-h follow-up of protein accumulation by the cells showed a significant augmentation in the protein-to-DNA ratio by 48 and 72 h of treatment (Fig. 2). The anabolic hormone triiodothyronine (T3, 5 n*M*) increased the protein-to-DNA ratio by 40–50% under similar experimental conditions (Fig. 2).

AII Effect on MHC Composition

When neonatal heart myocytes are grown in serum-free, T3-deficient medium, βMHC is constitutively expressed (Kessler-Icekson, 1988). Administration of AII to such cells increased the relative abundance of βMHC iso-mRNA by about 90%, whereas that of αMHC remained at background levels (*lanes C* and *A* in Fig. 3 *a*; *A*

in Fig. 4). The intensity of radioactive labeling of βMHC isoprotein was higher in AII-treated than in AII-untreated myocytes, whereas no detectable incorporation could be found in αMHC isoprotein (Fig. 3 *b*). As the stimulatory effect of AII was restricted to the βMHC, it did not modify the distribution of MHC phenotype; the neonatal myocytes retained βMHC as the predominant isoform (*lanes C* and *A* in Fig. 3 *c*).

We then tested for the ability of AII to shift the myocytes from α- to βMHC predominance, once they produced αMHC at the time of AII administration. This was achieved by a 24-h treatment with T3 (5 n*M*), followed by a 24-h T3-washout before an additional 48 h in 100 n*M* AII. The results demonstrate that AII could not downregulate the expression of αMHC and reestablish the predominance of βMHC iso-mRNA and isoprotein (T/A in Figs. 3,4). We conclude that the potency of AII to regulate the expression of MHC isoforms is restricted to the βMHC and is overridden by thyroid hormone.

Discussion

The central finding of this study is that the βMHC isogene is upregulated during AII-induced hypertrophy of beating myocytes from neonatal rat hearts. Despite the immature nature of these cells, which retain the capacity to divide and express fetal heart proteins, they are generally accepted as an adequate model for studies of myocardial cell hypertrophy in response to humoral and physical stimuli (e.g., Simpson et al., 1982; Simpson, 1985; Meidell et al., 1986; Aceto and Baker, 1990; Baker and Aceto, 1990; Komuro et al., 1990, 1995; Waspe et al., 1990; Bishopric et al., 1992; Sadoshima and Izumo, 1993a, 1993b; Sadoshima et al., 1993; Johnson et al., 1994). To allow the cells to hypertrophy, the cultures must be kept subconfluent, a condition that favors overgrowth by nonmyocytes. The standard measures preventing nonmyocyte overgrowth include differential plating, cytostatic agents, and serum-free medium. In many cases, these measures yield cleaner myocyte populations that are either quiescent or poorly contracting (Simpson, 1985; Waspe et al., 1990; Bishopric et al., 1992; Johnson et al., 1994). Because perpetual contractions are the normal presentation of cardiomyocytes *in vivo*, we restricted our study to spontaneously beating cultures, being aware that the basal rate of protein synthesis may be affected by the mechanical activity (McDermott et al., 1985; Johnson et al., 1994).

Previous studies on AII-induced hypertrophy showed that whereas embryonic chick myocytes responded to a single dose of AII, neonatal rat myocytes required multiple administrations to obtain a similar effect (Aceto and Baker, 1990; Baker and Aceto, 1990; Sadoshima and Izumo, 1993a). We describe conditions in which neonatal rat myocytes, like embryonic chick myocytes, undergo hypertrophy over 48 h after a single administration of AII. The overall increase in protein accumulation we observed (10–40%) was somewhat lower than that reported in multiple-administration experiments (40–50%, Sadoshima and Izumo, 1993a), a possible result of AII decrease with time. The effect of cell contraction, endogenous AII, or both could also lower the increment of AII-induced hypertrophy by raising basal protein synthesis. The concentration we used, 100 n*M*, yielded the maximal effect in our dose-response experiments (about 40% above the control). Although this concentration is much higher than the picomolar levels found in the circulation (Huang et al., 1989), the concentrations of locally generated AII in the vicinity of the myocytes *in vivo* are as yet unknown. Angiotensin II also stimulates the expression of angiotensinogen in cultured myocytes (Sadoshima and Izumo, 1993a), and it is possible that newly formed AII helps sustain the trophic effects of exogenously added AII over prolonged periods by positive-feedback regulation.

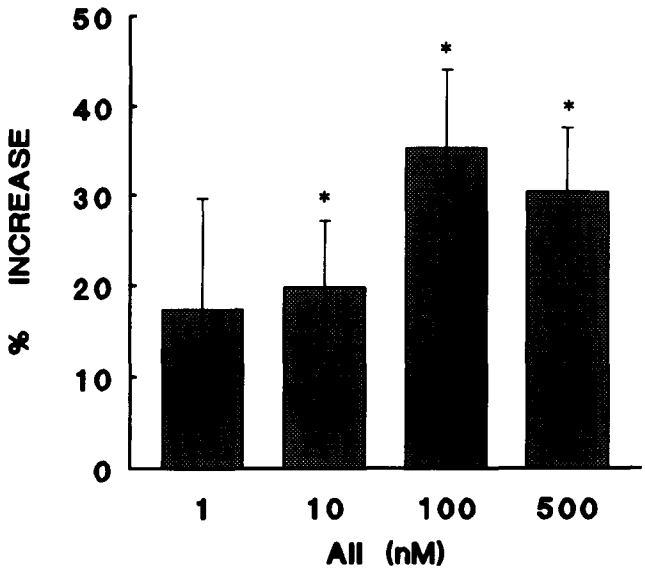

FIG. 1. Concentration dependence of protein accumulation. Four-d-old cultures grown as in protocol 2 (Materials and Methods) received 1–500 n*M* angiotensin II and [^{14}C]phenylalanine for 24 h. The percent increase in phenylalanine incorporation was calculated considering the untreated cultures as 100%. Each point summarizes 3–11 independent experiments. *Bars* indicate the mean ± SE; an *asterisk* indicates a value significantly different from that of untreated cultures ($P < 0.05$).

As mentioned earlier, AII-induced hypertrophy of cultured myocytes has been associated with increased amounts of rRNA and of several mRNA species encoding growth-regulating and tissue-spe-

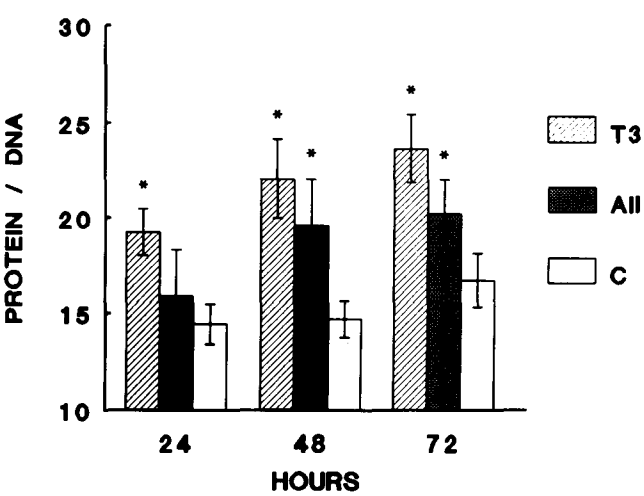

FIG. 2. Protein content in angiotensin II (AII)-treated myocytes. Four-d-old cultures grown as in protocol 1 (Materials and Methods) received either AII (100 n*M*), the anabolic hormone triiodothyronine (T3, 5 n*M*), or none. Cultures were removed for the determination of protein and DNA at 24, 48, and 72 h. Each point summarizes nine experiments; *Bars* indicate the mean ± SE; an *asterisk* indicates a value significantly different from that of untreated cultures (C) ($P < 0.05$).

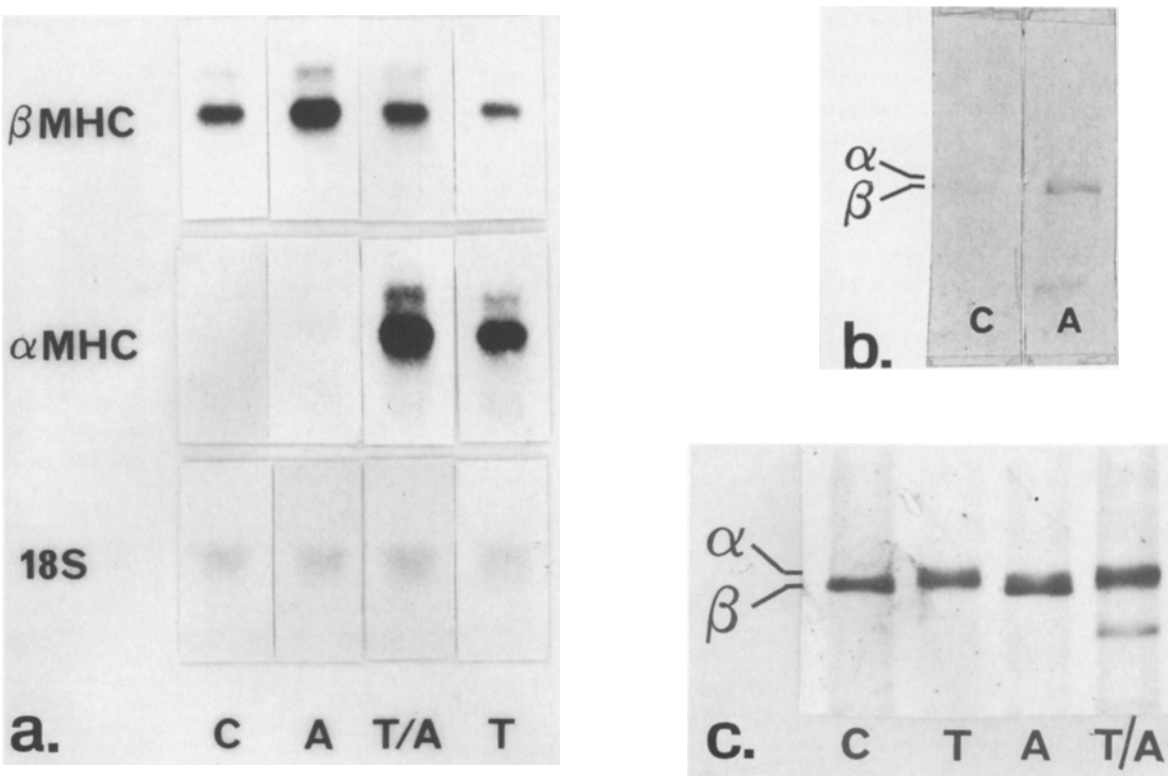

FIG. 3. AII effect on MHC iso-mRNA and isoprotein. (*a*) Autoradiograms of representative Northern blots. Identical lanes are shown of a membrane sequentially probed for αMHC-mRNA, βMHC-mRNA, and 18S-rRNA. Myocytes were incubated for 48 h with (A, T/A), or without (C,T) 100 n*M* AII. Part of the cultures were conditioned with 5 n*M* T3 and T3-washout (T, T/A) before AII treatment. (*b*) An autoradiogram of a dried gel showing radiolabeled MHCs from cultures incubated for 48 h with (*lane A*) or without (*lane C*) 100 n*M* AII in the presence of [^{14}C]phenylalanine. (*c*) MHC polypeptides from cultures treated as in (*a*), subjected to denaturing electrophoresis. Equal amounts of protein were loaded in each lane and stained with silverstain.

cific proteins. When we undertook a similar study with the α- and βMHC isogenes, we found that the abundance of the β-, not α-, MHC mRNA was increased by AII and that the neosynthesis of its protein was enhanced. The abundance of βMHC mRNA, estimated relative to that of rRNA, was nearly doubled by AII. We did not measure the absolute content of rRNA in the myocytes, yet we point out that a concomitant increase in the amount of rRNA means that the absolute elevation in βMHC mRNA might be higher than detected.

Preferential increase in βMHC iso-mRNA and isoprotein was shown before in α_1-adrenoceptor-stimulated neonatal rat myocytes (Waspe et al., 1990). Evidence was provided that activated protein kinase C induced an increase in transcription of the βMHC isogene which was mediated by the TEF-1 transcription factor (Kariya et al., 1991). Several reports attributed a central role to protein kinase C in the signal transduction pathways of AII-induced hypertrophy and gene regulation in cardiac myocytes (Baker and Aceto, 1990; Baker et al., 1992; Sadoshima and Izumo, 1993b). None of these studies explored the regulation of the βMHC isogenes. The detailed mechanism by which AII regulates the expression of the βMHC isogene remains to be elucidated.

In adult rats *in vivo*, AII acts on myocardial cells that coexpress α- and βMHC, αMHC being the predominant isoform. It is not clear, however, whether AII actively triggers a transition from α- to βMHC predominance. In the cultured myocytes, we found that AII could not potentiate the induction of β- and the deinduction of αMHC isogenes when the expression of αMHC had been induced by T3. The observed dominant effect of T3 on the pattern of MHC distribution is compatible with reports from studies on cardiac and skeletal muscles *in vivo*, showing that the influence of thyroid hormone overrides the biochemical changes associated with adaptational hypertrophy (Izumo et al., 1987; Ianuzzo et al., 1991).

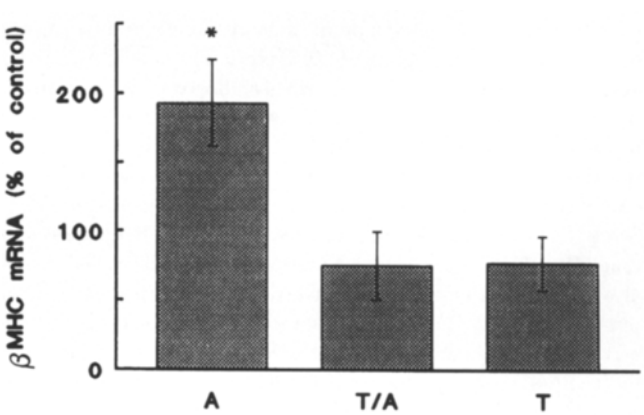

FIG. 4. Summary of densitometric quantifications of βMHC-mRNA. Symbols A, T, and T/A are as in Fig. 3. An *asterisk* indicates a value significantly different from that of untreated cultures ($P < 0.05$).

It is generally accepted that workload- and catecholamine-induced hypertrophies reactivate in the heart a 'fetal gene program' (reviewed by Chien et al., 1993; Komuro and Yazaki, 1993). Neonatal rat myocytes that are kept serum-free maintain the embryonic pattern of MHC distribution, the β isoform being constitutively expressed whereas the α isoform is nearly null (Kessler-Icekson, 1988). Therefore, the effect of AII on the βMHC isogene should be considered an upregulation rather than a reactivation of fetal gene expression. The same holds true for other markers of the embryonic heart such as α-skeletal actin and atrial natriuretic peptide which are upregulated, not reactivated, by AII in neonatal myocytes (Sadoshima et al., 1993; our unpublished data). The increase in βMHC expression may be viewed as part of a general enhancement in the production of pre-existing proteins and not necessarily as a return to the fetal gene program. The issue of whether AII signals the accumulation of every protein which is currently produced in the cells or is selective for the embryonic subset of cardiac isogenes remains open for further investigation.

Acknowledgments

This study was supported by grants from The Basic Research Foundation, administered by The Israeli Academy of Sciences and Humanities, and from The German–Israeli Foundation for Scientific Research and Development.

References

Aceto, J. F.; Baker, K. M. [Sar1]angiotensin II receptor mediated stimulation of protein synthesis in chick myocytes. Am. J. Physiol. 258:H806–H813; 1990.

Allen, I. S.; Cohen, N.; Dhallan, R., et al. Angiotensin II increases spontaneous contractile frequency and stimulates calcium current in cultured neonatal rat heart myocytes: insights into the underlying biochemical mechanisms. Circ. Res. 62:524–534; 1988.

Alpert, N. R.; Mulieri, L. A. Increased myothermal economy of isometric force generation in compensated cardiac hypertrophy induced by pulmonary artery constriction in the rabbit. Circ. Res. 50:491–500; 1982.

Baker, K. M.; Aceto, J. F. Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. Am. J. Physiol. 259:H610–H628; 1990.

Baker, K. M.; Booze, G. W.; Dostal, D. E. Cardiac actions of angiotensin II: role of an intracardiac renin-angiotensin system. Annu. Rev. Physiol. 54:227–241; 1992.

Bergman, M.; Cohen, F.; Schlesinger, H., et al. Effects of amiodarone on beating rate and Na-K-ATPase activity in cultured neonatal rat heart myocytes. Gen. Pharmacol. 26:285–290; 1995.

Bishopric, N. H.; Sato, B.; Webster, K. A. β-Adrenergic regulation of a myocardial actin gene via a cyclic AMP-independent pathway. J. Biol. Chem. 267:20932–20936; 1992.

Buttrick, P.; Malhotra, A.; Factor, S., et al. Effect of aging and hypertension on myosin biochemistry and gene expression in the rat heart. Circ. Res. 68:645–652; 1991.

Carrier, L.; Boheler, K. R.; Chassgne, C., et al. Expression of the sarcomeric actin isogenes in the rat heart with development and senescence. Cir. Res. 70:999–1005; 1992.

Chien, K. R.; Zhu, H.; Knowlton, K. U., et al. Transcriptional regulation during cardiac growth and development. Annu. Rev. Physiol. 55:77–95; 1993.

Chumczynski, P.; Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156–159; 1987.

Huang, H.; Baussant, T.; Reade, R., et al. Measurement of angiotensin II concentration in rat plasma: pathophysiological applications. Clin. Exp. Hyper. Theory Practice A11:1535–1548; 1989.

Ianuzzo, C. D.; Hamilton, N.; Li, B. Competitive control of myosin expression: hypertrophy vs hyperthyroidism. J. Appl. Physiol. 70:2328–2330; 1991.

Izumo, S.; Lompre, A. M.; Matsuoka, R., et al. Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy: interaction between hemodynamic and thyroid hormone induced signals. J. Clin. Invest. 79:970–977; 1987.

Johnson, T. B.; Kent, R. L.; Bubolz, B. A., et al. Electrical stimulation of contractile activity accelerates growth of cultured neonatal cardiocytes. Circ. Res. 74:448–459; 1994.

Kariya, K.; Karns, L. R.; Simpson, P. C. Expression of a constitutively activated mutant of the beta-isozyme of protein kinase C in cardiac myocytes stimulates the promoter of the beta-myosin heavy chain isogene. J. Biol. Chem. 266:10023–10026; 1991.

Kessler-Icekson, G.; Sperling, O.; Rotem, C., et al. Cardiomyocytes cultured in serum-free medium, growth and creatine kinase activity. Exp. Cell Res. 155:113–120; 1984.

Kessler-Icekson, G. Effect of tri-iodothyronine on cultured neonatal rat heart cells: beating rate, myosin subunits and CK-isozymes. J. Mol. Cell. Cardiol. 20:649–755; 1988.

Komuro, I.; Kaida, T.; Shibazaki, Y., et al. Stretching cardiomyocytes stimulates proto-oncogene expression. J. Biol. Chem. 265:3595–3598; 1990.

Komuro, I.; Yazaki, Y. Control of cardiac gene expression by mechanical stress. Annu. Rev. Physiol. 55:55–75; 1993.

Komuro, I.; Kudoh, S.; Zou, Y. Z., et al. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. Circ. Res. 77:258–265; 1995.

Labarca, C.; Paigen, K. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. 102:344–352; 1980.

Lompre, A. M.; Mercadier, J. J.; Wisnewsky, C., et al. Species- and age-dependent changes in the relative amounts of cardiac myosin isoenzymes in mammals. Dev. Biol. 84:286–290; 1981.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L., et al. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.

Lindpaintner, K.; Jin, M.; Wilhelm, M. J., et al. Intracardiac generation of angiotensin and its physiologic role. Circulation (Suppl. I) 77:18–23; 1988.

Mahdavi, V.; Periasamy, M.; Nadal-Ginard, B. Molecular characterization of two myosin heavy chain genes expressed in the adult heart. Nature (London) 297:659–664; 1982.

McDermott, P.; Daood, M.; Klein, I. Contraction regulates myosin synthesis and myosin content of cultured heart cells. Am. J. Physiol. 249:H763–H764; 1985.

Meidell, R. S.; Sen, A.; Henderson, S. A., et al. α1-Adrenergic stimulation of rat myocardial cells increases protein synthesis. Am. J. Physiol. 251:H1076–H1084; 1986.

Mendez, R. E.; Pfeffer, J. M.; Ortola, F. V., et al. Atrial natriuretic peptide transcription, storage, and release in rats with myocardial infarction. Am. J. Physiol. 253:H1449–H1455; 1987.

Mercadier, J. J.; Lompre, A. M.; Wisnewsky, C., et al. Myosin isoenzyme changes in several models of rat cardiac hypertrophy. Circ. Res. 49:525–532; 1981.

Sadoshima, J.; Izumo, S. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts, critical role of the AT_1 receptor subtype. Circ. Res. 73:413–423; 1993a.

Sadoshima, J.; Izumo, S. Signal transduction pathways of angiotensin II-induced c-fos gene expression in cardiac myocytes in vitro; roles of phospholipid-derived second messengers. Circ. Res. 73:424–438; 1993b.

Sadoshima, J.; Xu, Y.; Slayter, A. S., et al. Autocrine release of angiotensin II mediates stretch induced hypertrophy of cardiac myocytes in vitro. Cell 75:977–984; 1993.

Schorb, W.; Booze, G. W.; Dostal, D. E., et al. Angiotensin II is mitogenic in neonatal cardiac fibroblasts. Circ. Res. 72:1245–1254; 1993.

Schwartz, K.; Lecarpentier, Y.; Martin, J. L., et al. Myosin isoenzyme distribution correlates with speed of myocardial contraction. J. Mol. Cell. Cardiol. 13:1071–1075; 1981.

Simpson, P. Stimulation of hypertrophy of cultured neonatal rat heart cells through an alpha-1 adrenergic receptor and induction of beating through an alpha-1 and beta-1 adrenergic receptor interaction: evi-

dence for the independent regulation of growth and beating. Circ. Res. 56:884–894; 1985.

Simpson, P.; McGrath, A.; Savion, S. Myocyte hypertrophy in neonatal rat heart cultures and its regulation by serum and by catecholamines. Circ. Res. 51:787–801; 1982.

Timmermans, P. B. M. W. M.; Wong, P. C.; Chiu, A. T., et al. Nonpeptide angiotensin II receptor antagonists. Trends Pharmacol. Sci. 12:55–62; 1991.

Villareal, F. J.; Kim, N. N.; Ungab, G. D., et al. Identification of functional angiotensin II receptors on rat cardiac fibroblasts. Circulation 88:2849–2861; 1993.

Waspe, L.; Ordahl, C.; Simpson, P. C. The cardiac β-myosin heavy chain isogene is induced selectively in α_1-adrenergic receptor stimulated hypertrophy of cultured rat heart myocytes. J. Clin. Invest. 85:1206–1214; 1990.