Effect of Diabetes and Hypothyroidism on the Predominance of Cardiac Myosin Heavy Chains Synthesized in Vivo or in a Cell-free System*

(Received for publication, April 11, 1983)

Wolfgang H. Dillmann1, Alice Barriex, and George S. Reese

From the Division of Endocrinology, Department of Medicine, University of California, San Diego, California 92103

Rat cardiac ventricular myosins and RNA were prepared from normal, diabetic, insulin-treated diabetic, hypothyroid, and 3,3',5-triiodo-L-thyronine-treated hypothyroid rats. Myosin heavy chains isolated from purified myosin or synthesized in vitro from cardiac RNAs were subjected to partial protease digestion during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was found that peptide maps obtained from cardiac myosin heavy chains of hypothyroid and diabetic rats were identical but differed from the maps of myosin heavy chain from control and hormone-treated animals. The same results were obtained, whether the heavy chains were isolated from purified myosin synthesized in the intact heart or from translation products coded for by cardiac RNAs added to the modified reticulocyte lysate. These results indicate that the myosin heavy chain RNA species present in the hypothyroid heart is also expressed during insulin deficiency but differs from the species expressed in normal animals. The expression of the two myosin heavy chain RNA species found in the rat cardiac ventricle appears to be independently regulated by these two hormones.

In the rat, cardiac myosin can be separated into three isoforms on the basis of their electrophoretic mobilities (1-3). The three isoforms, designated V1, V2, and V3, following Hoh's nomenclature (1), appear to consist of identical pairs of light chain 1 and light chain 2, but differ in the composition of the myosin heavy chains (4, 5). Two identical heavy chains, MHCα,1 are found in V1; two identical heavy chains, MHCβ, are found in V3, and heterologous heavy chains, one MHCα and one MHCβ, are associated with V2 (5). The three forms of myosin exhibit different Ca2+-activated ATPase activities (1-3) and their relative abundance varies with the age (4, 6-10) and the hormonal status of the animals (1, 3, 11). 

* This work was done at the University of California, San Diego, and was supported by National Institutes of Health Grants HL25022 and HL27880. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

+ To whom correspondence should be addressed at University of California, San Diego Medical Center, Department of Medicine (H-811-C), 225 Dickinson Street, San Diego, CA 92103.

† The abbreviations used are: MHC, myosin heavy chain; T3, 3,3',5-triiodo-L-thyronine; T4, L-thyroxine, SDS, sodium dodecyl sulfate.

The highest Ca2+-activated ATPase activity is the predominant form in young, healthy adults; V3 with the lowest Ca2+-activated ATPase activity is the major embryonic form. An isozyme indistinguishable from embryonic V2 myosin by electrophoretic migration is also present in small amounts in the young adult and becomes the predominant form in animals rendered severely hypothyroid or diabetic (1, 3, 11).

A minimum of 5 MHC genes are present in the rat and at least 2 are expressed in the heart (12, 13). Indeed, Schwartz et al. (7) were able to demonstrate by specific antibodies and chymotrypsin mapping that MHC expressed in adult hypothalamic heart tissue was identical to the MHC expressed in embryonic heart but differed from the MHC expressed in normal young hearts. In addition, since two partial cDNA clones exhibiting slightly different nucleotide sequences have been obtained from young adult rat cardiac mRNA (12), at least two different MHC genes are expressed in the heart of young rats. These two clones hybridized only weakly with embryonic cardiac mRNA, suggesting that either two allelic forms of MHCα are expressed in young adults as proposed by the authors, or MHCα expressed in young adults is not identical to that expressed in embryos. In the rabbit heart, myosin can also be expressed as isoforms and it has been established that MHCs present in V1 and V3 are coded for by two different mRNAs (14). Different MHCs giving rise to different peptide maps, however, have been reported to be generated by post-translational modification of a single species of MHC in the chicken skeletal muscle (15). Therefore, although electrophoretically identical isoforms of native myosin V3 can be isolated from hypothyroid or diabetic heart in the rat, it is not evident that these two pathological conditions, induced by the lack of hormones that have very different mechanisms of action, result in the presence of a common MHC in myosin V3.

In this report, we present evidence that MHCs translated from RNA isolated from hypothyroid and diabetic animals are identical. They differ, however, from MHC coded for by RNA isolated from the heart of control animals or hypothyroid animals treated with thyroid hormone and diabetic animals treated with insulin. Thus, although the action of T3 is mediated via nuclear receptors while that of insulin is via membrane receptors, these two hormones either directly or indirectly appear to regulate the expression of the two cardiac mRNAs which code for MHCα and MHCβ.

MATERIALS AND METHODS

Animal Preparations—Male Sprague-Dawley rats weighing between 200 and 250 g were used in all experiments. Hypothyroidism was induced as previously described (16). Hypothyroid animals stopped gaining weight and had plasma T4 levels of 1.82 ± 0.5 µg/100 ml and plasma T3 levels of 30 ± 16 ng/100 ml. Normal rats had plasma T4 levels of 5.1 ± 0.8 µg/100 ml and plasma T3 levels of 69 ± 16 ng/100 ml. Plasma T4 was analyzed by competitive protein binding assay (17) and T3 was determined by radioimmunoassay (18). Four weeks after the onset of hypothyroidism, half of the animals received daily injections of 0.4 µg of T3/100 g of body weight while the remaining animals were kept completely hypothyroid. Diabetes was induced by administration of 65 mg of streptozotocin/kg body weight. Blood glucose levels in control animals were 125 ± 18 mg/dl and in diabetic animals 435 ± 30 mg/dl. Blood glucose levels did not change significantly during the course of the experiments. Three days after diabetes induction, half of the animals were given daily intramuscular injections of 2 units of protamine zinc insulin/100 g body weight for three weeks. The other half were kept for the
same length of time without insulin administration. Control animals matched for age were included in the study. Animals were killed by exsanguination. Fresh hearts pooled from 2 to 3 animals were used for each RNA preparation. Individual hearts frozen in liquid nitrogen and stored at −80 °C for 2 to 8 days were used for the preparation of myosin.

**Peptide Map of Myosin Heavy Chain—**Myosin was purified and Ca^{2+}-activated myosin ATPase was measured as previously described (19). Peptide mapping of myosin heavy chain from the 5 groups of animals was performed as described by Cleveland et al. (20). Myosin heavy chain was either digested in solution without prior purification by incubating purified myosin with *Staphylococcus aureus* vata protease (Miles Laboratories) as indicated in Fig. 1 or was prepurified from either myosin or total translation products on 5% polyacrylamide gels using the system described by Laemmli (21).

Minor modifications were introduced in the technique described by Cleveland et al. (20). Polyacrylamide slab gels cross-linked with AcrylAide and covalently bound to Gelbond PAG films (FMC Corp.) were used to analyze the polypeptides after myosin digestion in solution. Polyacrylamide slab gels, also cross-linked with AcrylAide and covalently bound to Gelbond PAG films (FMC Corp.) were cut and incubated in Cleveland buffer (20) for 2 h and applied to a second gel containing 12.5% polyacrylamide cross-linked with bisacrylamide. Because of the large molecular mass of MHC compared to that of the proteolytic enzymes, segregation of intact substrate and enzymes already occurred in the stacking gels. Therefore, the enzyme solutions layered onto the preparative gel pieces containing MHC was increased to 30 μl and the length of the 3% polyacrylamide stacking gel was reduced to 0.5 cm to prevent further digestion of peptides generated from MHC digestion during migration through the preparative gel piece. The enzyme solutions were overlaid with 10-μl 10% boric acid containing 0.01% bromophenol blue and the gels were run at a constant current of 4 mA/gel until the tracking dye reached the stacking gel and at 15 mA/gel thereafter. The concentrations of *S. aureus* vata protease and chymotrypsin were as indicated in the text. After electrophoresis, the peptides were stained with Coomassie brilliant blue R 250 or silver (23).

RNA Preparation and Translation—RNA was prepared from fresh hearts as previously described (16). Unfractionated RNA was translated in the nuclease-treated rabbit reticuloocyte lysate prepared in our laboratory according to Pelham and Jackson (22).

Ten to 15 μCi of [35S]methionine (900-1400 Ci/mmol) and 1-2 μg of RNA were added per 10-μl reaction. After 1-h incubation at 30 °C, the reactions were stopped by the addition of 1 μg of RNase A and 20 μg of methionine. After 15 min at room temperature, 1 μl dithiothreitol was added to a final concentration of 50 mM. Incorporation of [35S]methionine into acid-insoluble material was measured by the method of Mans and Novelli (24). [35S]Methionine incorporation into MHC was quantitated as follows. To aliquots containing 100,000 acid-precipitable cpm, were added 10 μg of purified myosin. MHC was then separated from other translation products by electrophoresis on a 10% polyacrylamide-SDS gel (21). Proteins were visualized by staining, and the MHC band was cut out, thoroughly destained in 45% methanol, 10% acetic acid, dried, and counted in 5 ml of Betamax (WestChem). Translation aliquots containing the same amount of [35S]MHC were used for peptide mapping. After the addition of 10 μg of purified myosin to each sample, mapping was performed as described for purified myosin. At the end of electrophoresis, the gels were prepared for fluorography as previously described (16).

**RESULTS**

The hormonal status of hypothyroid and diabetic animals was documented as previously described (3, 16). Administration of 0.4 μg of T3/100 g body weight/day to diabetic rats and 2 units of protamine zinc insulin/100 g body weight/day to diabetic animals normalized Ca^{2+}-activated myosin ATPase activity and the myosin isoenzyme distribution pattern.

The predominance of MHC among total cardiac proteins was similar for animals of different hormonal status; the mobility of MHC in SDS-polyacrylamide slab gels, however, appeared to be very slightly increased when myosin was isolated from hormone-deficient hearts (Fig. 1). There was no difference between the 5 myosin preparations used, although a slight increase in minor contaminants may be present in the myosin isolated from insulin-treated diabetic animals (Fig. 1, I). Limited *S. aureus* V8 protease digestion of myosin isolated from the hearts of normal and hormone-injected rats led to peptide maps different from those obtained from myosin.
of hypothyroid and diabetic animals (Fig. 1). Six peptides characteristic of myosin isolated from the heart of normal (C), T₃-treated (T), or insulin-treated (I) rats were seen at the lower protease concentration (0.08 pg). None of these peptides were present in the digests of either diabetic (D) or hypothyroid (H) myosin; they were replaced by seven new peptides not seen or only faintly visible in the normal and hormone-treated myosin. Unique peptides characteristic of either diabetic and hypothyroid or control and hormone-treated myosin were observed at the two concentrations of V₈ protease. The two lowest peptides seen at the higher protease concentration may not originate from MHC since myosin light chain 2 was also digested at this protease concentration. When MHC was first separated from other myosin subunits and digestion was allowed to proceed during electrophoresis, a smaller set of peptides was obtained even at a protease concentration which resulted in only partial digestion of MHC (Fig. 2). However, as observed for myosin digested in solution, peptides common to the digests of control (C) and hormone-treated (T, I) MHC, indicated by arrows on the left, were not present in the digests of diabetic (D) and hypothyroid (H) MHC, while at least 4 peptides were unique for diabetic and hypothyroid MHC. Similar results were obtained when V₈ protease was replaced by chymotrypsin; one set of peptides common to control (C) and hormone-treated (T, I) MHC (Fig. 3, arrows on the left) were not observed in the hormone-deficient myosins (D, H), and 5 peptides seen in the latter were not present or barely visible in the control group (Fig. 3, arrows on the right). It should be mentioned that the control MHC map differs from the hormone-treated MHC maps by 2 peptides (broken arrows).

To determine whether the differences in the peptide maps of MHC resulted from post-translational modifications of a single MHC species or from two MHC polypeptides coded for by different mRNA species, RNAs isolated from the heart of normal, hormone-deficient, and hormone-treated animals were translated in a cell-free system, and [³⁵S]methionine-labeled MHC was subjected to limited chymotrypsin or S. aureus V₈ protease digestion. The distribution of total translational products coded for by RNA isolated from the five groups of animals was analyzed by SDS-polyacrylamide gel electrophoresis. MHC migrated with the same mobility in each group and represents one of the major translational products (data not shown). [³⁵S]Methionine present in MHC was measured as described under "Materials and Methods" and aliquots containing the same amounts of radioactive MHC were used for limited protease digestion. Results presented in Fig. 4 (S. aureus V₈ protease digests) and Fig. 5 (chymotrypsin digests) show that peptide maps obtained from diabetic and hypothyroid MHC are identical and differ from peptide maps obtained from the control and hormone-treated MHC. It should be noted that the T₃-treated sample appears to contain peptides characteristic of both types of MHC, indicating that the dose of T₃ used to treat animals in this particular experiment was not sufficient to completely normalize MHC mRNA.

**DISCUSSION**

This report demonstrates for the first time that mRNA isolated from the heart of hypothyroid and diabetic rats synthesizes an identical MHC which differs from MHC coded...
for by cardiac RNA isolated from normal or hormone-treated animals. The same MHC RNA species must therefore be present in hypothyroid and diabetic ventricles, and must differ from mRNA present in the heart of normal and hormone-treated animals. The results indicate that at least two different mRNA species coding for two myosin heavy chains must be expressed in the rat heart, as it has been shown for the rabbit heart (14). Although others have reported that rat cardiac MHC expressed in hypothyroid rats differs from that occurring in normal rats (1, 7), the possibility that post-translational modification of a single MHC species resulted in the observed differences in peptide maps could not be excluded in these reports. In the chicken fast twitch muscle, for example, MHCs having different peptide maps are coded for by a single mRNA species (15). In the present study, different maps were obtained from hormone-deficient and normal MHCs whether MHC was obtained from intact myosin purified from cardiac ventricles or from translation products synthesized in vitro from cardiac RNAs. The slight difference seen between control and hormone-treated myosin digests only when chymotrypsin was used to digest MHC synthesized in vivo may indicate different levels of post-translational modification in the 2 groups.

MHC is a large polypeptide which represents a significant fraction of the total cardiac proteins. In comparing peptide maps of MHCs obtained from hormone-deficient and control tissues, we have assumed that the prominent band, having a similar peptide map of MHCs obtained from hormone-deficient and control tissues, we have assumed that the prominent band, having a similar

isoenzyme distributions in hypothyroid rats do not do so in diabetic animals (29). The lower $T_2$ and $T_3$ levels of diabetic rats therefore cannot explain the myosin $V_2$ predominance which occurs in diabetes.

Acknowledgments—We gratefully acknowledge Dr. Nicholas Alexander for performing the $T_2$ and $T_3$ assays. We thank Stuart Berry and Chris Lehman for their technical assistance and Sonja VanBuuren for her assistance in preparation of this manuscript.

REFERENCES

1. Hoh, J. F. Y., McGrath, P. A., and Hale, P. T. (1978) J. Mol. Cell. Cardiol. 10, 1053-1076
2. Lompré, A. M., Schwartz, K., d’Albis, A., Lacombe, G., Van Thiem, N., and Swnyghedau, B. (1979) Nature (Lond.) 282, 105-107
3. Dillmann, W. H. (1980) Diabetes 29, 579-582
4. Gorza, L., Faletto, P., Pessina, A. C., Sartore, S., and Schiaffino, S. (1981) Circ. Res. 49, 1003-1009
5. Hoh, J. F. Y., Yeoh, G. P. S., Thomas, M. A. W., and Higgonbotom, L. (1979) FEBS Lett. 97, 330-334
6. Klotz, C., Swnyghedau, B., Mendes, H., Marotte, F., and Leger, J. J. (1981) Eur. J. Biochem. 115, 415-421
7. Schwartz, K., Lompré, A., Bouveret, P., Wisnewsky, C., and Whalen, R. G. (1983) Nature (Lond.) 307, 1415-1418
8. Whalen, R. G., and Sell, S. M. (1980) Nature (Lond.) 286, 731-733
9. Lompré, A. M., Mercadier, J. J., Wisnewsky, C., Bouveret, P., Pantaloni, C., d’Albis, A., and Schwartz, K. (1981) Dev. Biol. 84, 286-290
10. Mercadier, J. J., Lompré, A. M., Wisnewsky, C., Samuel, J. L., Bercovici, J., Swnyghedau, B., and Schwartz, K. (1981) Circ. Res. 49, 525-532
11. Malhotra, A., Penparghul, S., Fein, F. S., Sonnenblick, E. H., and Scheuer, J. (1983) Circ. Res. 49, 1243-1251
12. Nudel, U., Katoff, D., Carmon, Y., Zevin-Sonkin, D., Levi, Z., Shaul, Y., Shani, M., and Yaffe, D. (1980) Biochim. Biophys. Acta 602, 2133-2146
13. Mahdavi, V., Periasamy, M., and Nadal-Ginard, B. (1982) Nature (Lond.) 297, 650-664
14. Shina, A., Umeda, P. K., Kavinsky, C. J., Rajamani-Kumar, C., Hsu, H. J., Jakovicic, C., and Schiaffino, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 79, 5847-5851
15. Bandman, E., Matsuda, R., and Strohbr, R. C. (1982) Cell 29, 645-650
16. Dillmann, W. H., Barrieux, A., Neeley, W. E., and Contreras, P. (1983) J. Biol. Chem. 258, 7738-7745
17. Alexander, N. M., and Jennings, J. F. (1974) Clin. Chem. 20, 553-559
18. Alexander, N. M., and Jennings, J. F. (1974) Clin. Chem. 20, 1353-1361
19. Bahn, A. K., and Malhotra, A. (1976) Arch. Biochem. Biophys. 174, 24-35
20. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102-1106
21. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-688
22. Feibehm, H. R. B., and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247-256
23. Morrissey, J. H. (1981) Anior. Biochem. 117, 307-310
24. Mans, R. J., and Novelli, G. D. (1981) Arch. Biochem. Biophys. 194, 48-53
25. Chizzonite, R. A., Everett, A. W., Clark, W. A., Jakovyicic, S., Schiaffino, S., and Zalk, R. (1982) J. Biol. Chem. 257, 2056-2065
26. Rozeck, C. E., and Davidson, N. (1985) Cell 52, 23-34
27. Wydro, R. M., Nagyaver, H. T., Gubitov, R. M., and Nadal-Ginard, B. (1985) J. Biol. Chem. 258, 670-675
28. Samuel, J.-L., Rappaport, L., Mercadier, J.-J., Lompré, A. M., Sartore, T., Triban, C., Schiaffino, S., and Schwartz, K. (1983) Circ. Res. 52, 200-209
29. Dillmann, W. H. (1982) Metabolism 31, 199-203
Effect of diabetes and hypothyroidism on the predominance of cardiac myosin heavy chains synthesized in vivo or in a cell-free system.
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J. Biol. Chem. 1984, 259:2035-2038.

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