PRELIMINARY NOTE

BBA 41155

Effect of L-thyroxine on the primary structure of cardiac myosin

The hyperthyroid myocardium differs from the normal myocardium in cardiovascular dynamics and in muscle mass. By measurement of the rate of L-[1-^{14}C]leucine incorporation into tissue proteins of intact animals, MICHELS *et al.*[1] have demonstrated that thyroxine stimulates rat heart protein biosynthesis. GOODKIND *et al.*[2] recently showed that the hyperthyroid guinea pig has both increased myocardial contractility and increased cardiac myosin ATPase activity. We have examined cardiac myosin from hyperthyroid guinea pigs for modifications of the protein structure at the primary level. In this report we demonstrate that cardiac myosin from hyperthyroid guinea pigs has a special amino acid composition which may explain its relatively high helical content and altered enzymatic activity.

Hyperthyroidism was produced (L-thyroxine, intraperitoneally 0.25 mg/kg per day, 8 days) in 13 groups (containing 5 animals each) of male, Hartley strain guinea pigs. Control groups received similar volumes of saline over the same time period. Isometric contraction measurements demonstrated the presence of the effects of hyperthyroidism[2] on the myocardium of the experimental animals. Ventricular myosin was prepared by the LiCl–$(NH_4)_2SO_4$ fractionation technique described by LUCHI *et al.*[3]. Myosin ATPase activity (10^{-7} moles P/mg protein per min) determined as previously described[3], rose significantly in hyperthyroidism from 8.2 ± 0.7 (control, mean $\pm$ 1 S.D.) to 10.8 ± 1.3 ($P < 0.001$). The ATPase activity of normal cardiac myosin is similar to that of myosin from red (slow) skeletal muscle. Hyperthyroidism, however, appears to affect the transformation of cardiac myosin to an enzyme having an ATPase activity comparable to that of myosin from the white (fast) skeletal muscle prepared by our techniques. The extent of helix configuration in myosin was determined from optical rotation measurements at 233 mμ (ref. 4). Myosin samples (in KCl–borate buffer, pH 6.8) from five groups of control and experimental animals were analyzed. The helical content rose significantly in hyperthyroidism from 43 ± 3 % (control) to 55 ± 1 % ($P < 0.001$). The amino acid composition was obtained by the method of MOORE AND STEIN[5] utilizing a Beckman Model 120 C amino acid analyzer. Acid hydrolysis of myosin was carried out at 110° for 24, 48 and 72 h. Table I shows the amino acid content of myosin samples obtained from 15 pooled euthyroid and 20 pooled hyperthyroid ventricles. The amino acids in Table I have been arranged according to the classification of BLOOM *et al.*[6] into promoting and disturbing types with respect to helix formation. More recent studies[7] have shown that this classification does at least in part apply to the complex sequences found in proteins. Table I shows that the helical content of myosin from hyperthyroid animals can be interpreted as the result of its relatively low content of the helix disturbing amino acids threonine

and serine and its relatively high content of the helix-promoting amino acids lysine and glutamic acid.

TABLE I

AMINO ACID COMPOSITION OF CARDIAC MYOSIN FROM EUTHYROID AND HYPERTHYROID GUINEA PIGS

Amino acid	*Moles amino acid per 10^5 g myosin**	
	Euthyroid	*Hyperthyroid*
α-Helix promoting		
Lysine	87 ± 1	94 ± 3***
Histidine	17 ± 1	17 ± 1
Arginine	49 ± 1	50 ± 1
Aspartic acid	84 ± 1	89 ± 4
Glutamic acid	150 ± 3	162 ± 7§
Alanine	67 ± 2	72 ± 4
Methionine	21 ± 1	22 ± 1
Leucine	80 ± 2	85 ± 5
Tyrosine	16 ± 1	16 ± 1
Phenylalanine	28 ± 1	30 ± 3
Glycine	35 ± 1	35 ± 2
α-Helix disturbing		
Threonine**	40 ± 1	36 ± 1***
Serine**	38 ± 1	34 ± 1***
Proline	24 ± 1	23 ± 1
Valine	36 ± 1	38 ± 3
Isoleucine	36 ± 1	37 ± 3

* Mean ± 1 S.D. of 24, 48 and 72 h hydrolysis values, except as noted.
** Values extrapolated to zero time to correct for hydrolytic destruction.
*** Significantly different from euthyroid values, $P < 0.01$.
§ Significantly different from euthyroid values, $P < 0.05$.

In summary, we suggest that the administration of thyroxine to guinea pigs stimulates the synthesis of a new type of cardiac myosin or a new protein which is intimately associated with the cardiac myosin. At the present, at least three physicochemical properties of this myosin have been shown to be different from myosin isolated from euthyroid guinea pigs. These are the ATPase activity, the helical content and the amino acid composition.

Department of Medical Research,
Veterans Administration Hospital,
Iowa City, Iowa 52240 (U.S.A.)

P. T. THYRUM
E. M. KRITCHER
R. J. LUCHI

1 R. MICHELS, J. CASON AND L. SOKOLOFF, *Science*, 140 (1963) 1417.
2 M. J. GOODKIND, G. E. DAMBACH AND R. J. LUCHI, *J. Clin. Invest.*, 48 (1969) 30a.
3 R. J. LUCHI, E. M. KRITCHER AND H. L. CONN, JR., *Circulation Res.*, 16 (1965) 74.
4 R. J. LUCHI, E. M. KRITCHER AND P. T. THYRUM, *Circulation Res.*, 24 (1969) 513.
5 S. MOORE AND W. H. STEIN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 6, Academic Press, New York, 1963, p. 819.
6 S. M. BLOOM, G. D. FASMAN, C. DE LOZÉ AND E. R. BLOUT, *J. Am. Chem. Soc.*, 84 (1962) 458.
7 B. H. HAVSTEEN, *J. Theoret. Biol.*, 10 (1966) 1.

Received December 5th, 1969