MAINTENANCE OF PROTEIN SYNTHESIS IN HEARTS OF DIABETIC ANIMALS*

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SUMMARY

Skeletal muscle of diabetic animals contained increased numbers of ribosomal subunits and decreased polysomes. However, numbers of polysomes and subunits in heart muscle of diabetic rats were the same as controls. Incorporation of ^{14}C-phenylalanine into protein of diabetic hearts occurred at the control rate When control hearts were perfused, in vitro, with medium containing glucose and normal plasma levels of amino acids, polysomes decreased and subunits increased. These changes were prevented by addition of either insulin or palmitate suggesting that these factors facilitated peptide-chain initiation in heart muscle.

Incorporation of ^{14}C-amino acids into protein was inhibited in skeletal muscle of diabetic animals (1,2). Untreated diabetic animals were characterized by wasting of skeletal muscle together with a negative nitrogen balance (3,4). On the other hand, untreated diabetes has not been associated with cardiac atrophy or acute congestive heart failure. These observations suggested that protein synthesis in heart and skeletal muscle was effected in different ways in insulin-deficient animals. The present studies indicate that protein synthesis in hearts of diabetic animals proceeded at control rates due to a stimulatory effect of long-chain fatty acid on amino acid incorporation and peptide-chain initiation.

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EXPERIMENTAL PROCEDURE

Heart and psoas muscles were removed from fed rats that had been anaesthetized with Nembutal. Diabetes was induced by intravenous injection of alloxan (60 mg/kg) 48 hours prior to death. Hearts were perfused by a modified Langendorff technique (5) with Krebs-Henseleit bicarbonate buffer gassed with O_2:CO_2 (95:5%) that contained normal plasma levels of amino acids, ^{14}C-phenylalanine (0.25 μC/μmole) and glucose (15 mM). In some experiments, bovine serum albumin (3%) or 1.2 mM palmitic acid and albumin were added to the buffer. At the end of perfusion, incorporation of ^{14}C-phenylalanine into heart protein and ^{14}C-phenylalanine space were determined (6). Glucose uptake was determined by measuring disappearance of glucose from the perfusion medium (7). Sucrose gradient analysis of heart and psoas ribosomes was performed using an 8000 g supernatant of muscle homogenate. Hearts were homogenized in a solution containing 0.25 M KCl, 2 mM $MgCl_2$, and 10 mM Tris buffer, pH 7.4. Exponential sucrose gradients were formed in tubes of an SW-40 rotor using 15% and 2 M sucrose dissolved in homogenization buffer (8). Following centrifugation at 40,000 rpm for 3-1/4 or 15 hrs., gradients were monitored by pumping contents of the tube through the flow cell of a Gilford recording spectrophotometer.

RESULTS

Levels of polysomes and ribosomal subunits are influenced by rates of initiation and elongation of peptide chains. Low levels of subunits result when initiation is rapid relative to elongation. As seen in Fig. 1, increased numbers of subunits and decreased polysomes were found in psoas muscles of diabetic rats. Changes in numbers of subunits were seen best in sucrose

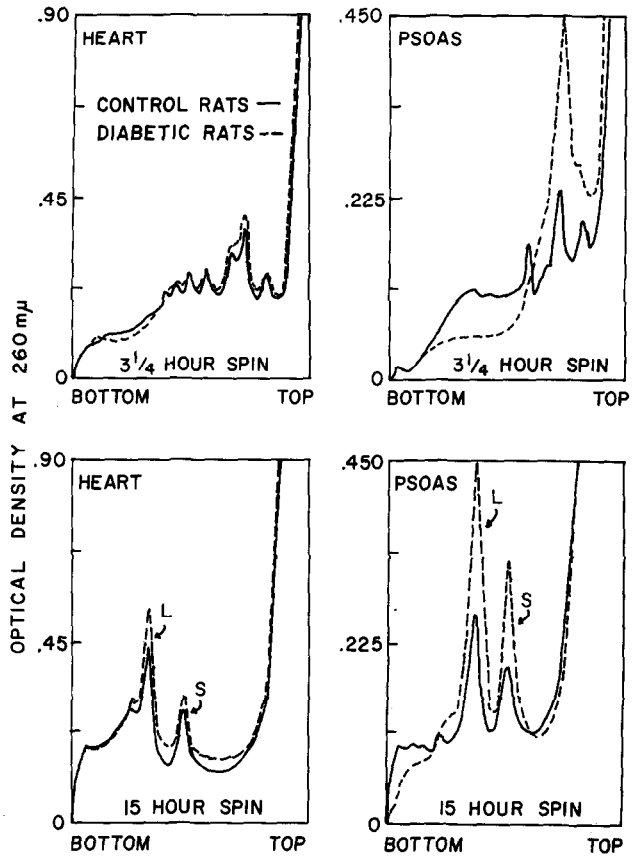

Fig. 1: Effect of diabetes on ribosomal aggregation in heart and skeletal muscle. Muscle was homogenized and sucrose gradient analysis carried out as described in Experimental Procedure. This experiment was repeated five times with similar results.

Subunit peaks were identified by collecting peaks from gradients spun for 15 hrs. Subunits were collected by diluting sucrose solutions with homogenization medium followed by centrifugation for 20 hrs. at 50,000 rpm in a 50.1 rotor. RNA was extracted from pellets (9) and analyzed on linear sucrose gradients. The large subunit peak (L) contained 28 S RNA together with a small amount of 18 S RNA. The small subunit peak (S) contained 18 S RNA together with a small amount of 28 S RNA.

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gradients spun for 15 hrs. Since amino acid incorporation was inhibited in diabetic muscle (1,2), these changes suggest that a block in peptide-chain initiation was involved in the inhibition. In contrast, hearts of diabetic animals contained

Table I. Effects of diabetes, insulin and palmitate on ^{14}C-phenylalanine incorporation, ^{14}C-phenylalanine space and glucose uptake.

Heart	^{14}C-phenylalanine incorporation cpm/mg	^{14}C-phenylalanine space ml/g	Glucose Uptake μmoles/g/hr.
Perfused 1 hour with buffer containing normal plasma amino acid levels and glucose (15 mM)			
Control	94 ± 2 (12)[a]	0.78 ± .02[a]	26.1 ± 9
Diabetic	94 ± 3 (10)	0.88 ± .02[b]	-8.3 ± 8[c]
Control + Insulin	117 ± 8 (4)[b]	0.73 ± .01	64.3 ± 2.1[c]
Perfused 3 hours with buffer containing 3% albumin, normal plasma amino acid levels and glucose (15 mM)			
Control	302 ± 7 (10)	0.82 ± .01	--
Control + Palmitate	355 ± 14[c] (10)	0.79 ± .02	--

Hearts were perfused as described in Experimental Procedure. The number of hearts is indicated by the figure in parenthesis. a = mean ± S.E.M.; b = $p < .02$ vs control; c = $p < .01$ vs control

the same numbers of polysomes and subunits as controls. This result could indicate that rates of chain initiation and elongation were unchanged in diabetic hearts or that the rate of protein synthesis was inhibited due to a block in chain elongation. As seen in Table I, incorporation of ^{14}C-phenylalanine into heart protein occurred at control rates in diabetic hearts when perfused *in vitro*. Distribution of ^{14}C-phenylalanine within the heart was increased somewhat, but uptake of glucose was completely inhibited. These observations indicated that amino acid incorporation and ribosomal activity were protected in hearts of insulin-deficient animals but that this protection was not shared by skeletal muscle.

Severe diabetes has been associated with increased levels of plasma FFA and increased fatty acid utilization by heart muscle (10). As seen in Fig. 2., perfusion of control hearts with buffer containing glucose and amino acids resulted in

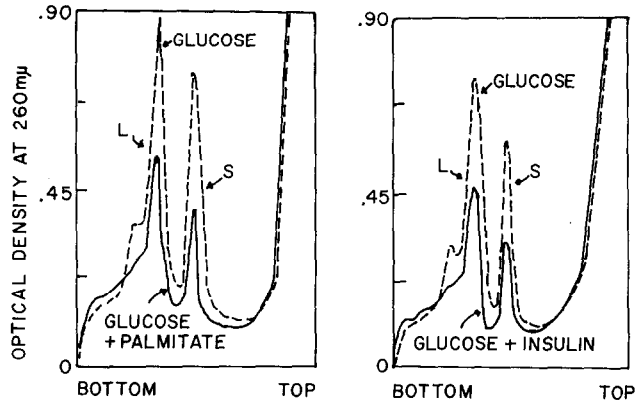

Fig. 2: Effect of insulin and palmitate on ribosomal aggregation. Hearts were removed from rats fasted overnight and perfused as described in Experimental Procedure. When the effect of insulin was studied, hearts were perfused for one hour with buffer containing normal plasma levels of all amino acids and glucose (15 mM). When the effect of palmitate was investigated, hearts were perfused for three hours with buffer containing 3% fat-free bovine serum albumin, normal plasma levels of all amino acids, and glucose. Sucrose gradients were spun for 15 hours. The experiments were repeated 3 to 6 times with similar results.

appearance of large numbers of subunits and decreased polysomes, suggesting that a block in peptide-chain initiation had developed during perfusion. Inclusion of palmitate resulted in low levels of subunits and more polysomes. As also seen in Fig. 2, insulin prevented disaggregation of polysomes. Since both insulin and fatty acid increased incorporation of ^{14}C-phenylalanine into heart protein (Table I), changes in ribosomal aggregation suggested that both of these factors facilitated peptide-chain initiation in heart muscle.

DISCUSSION

Protein synthesis in muscle tissue is thought to involve a cyclic process in which ribosomal subunits associate with mRNA, tRNA and other factors to form polysomes (11). When translation of mRNA is finished, the peptide chain, mRNA and subunits dis-

sociate to complete the cycle. Factors involved in controlling the ribosome cycle are poorly understood. In the present study, the cycle has been considered to consist of two groups of reactions, those involved in 1) chain-initiation and 2) chain-elongation. If rates of amino acid incorporation and numbers of subunits and polysomes are known, effects of various factors on these reactions can be deduced. Using this approach, peptide chain initiation appeared to be inhibited in skeletal muscle of diabetic rats since rates of amino acid incorporation were reduced (1,2) in association with increased subunits and decreased polysomes. This defect may be related to the impaired activity of ribosomes isolated from diabetic muscle (12,13). In contrast, protein synthesis in heart muscle of diabetic rats appeared to be unimpaired.

When control hearts were perfused *in vitro* with buffer containing glucose and amino acids, rates of amino acid incorporation declined, numbers of subunits increased, and polysomes fell. These findings indicated that a block in initiation had developed, perhaps as a result of depletion of fatty substrates and hormones during perfusion. In other experiments, intracellular levels of amino acids were measured in perfused hearts and found to be maintained at *in vivo* concentrations. Development of the block in initiation could be prevented by inclusion of either insulin or palmitate in the perfusate. Mechanisms of the stimulatory effects of these factors on initiation are unknown, but may involve regulation through changes in intracellular levels of metabolites. Unimpaired protein synthesis in hearts of diabetic rats could have depended upon the ability of fatty acids to facilitate peptide-chain initiation.

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