Vol. 138, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1986 Pages 374-380

EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES AND INSULIN TREATMENT ON THE DEGRADATION OF HEXOKINASE II IN THE SKELETAL MUSCLE OF THE RAT*

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Received May 23, 1986

SUMMARY. The rate of degradation of hexokinase II and the general pool of cytosolic proteins was measured in the skeletal muscle of normal, streptozotocin-diabetic, and insulin-treated streptozotocin-diabetic rats. The rate constant for hexokinase II degradation increases by a factor of 3 in the diabetic as compared with the normal animal. There were analogous alterations observed for the general pool of cytosolic proteins; however, the changes were much more subtle. Insulin treatment restores the increased degradative rate to normal levels in both classes of muscle proteins.

INTRODUCTION. Several investigations (1-4) have shown that there is a decrease in hexokinase II activity in the muscle tissue of the streptozotocin-diabetic rat and that, upon treatment with insulin, the activity is restored to normal levels. It is also known that there is an increase in overall protein degradation in the diabetic state (see reference 5 for review) and that, upon treatment with insulin this effect is reversed. In 1982, we reported an increase in the rate of hexokinase II degradation in skeletal muscle of the streptozotocin-induced diabetic rat (6). Henson et al. (7) reported that incubation of fat pads with insulin did not tend to stabilize hexokinase II against degradation any more than that of other cytosolic proteins. Rose and Warms (8) suggested that the loss of stabilization factors might contribute to the decrease in hexokinase II activity in insulin-sensitive tissues. They found that hexokinase II, which is readily oxidized in the absence of glucose *in vitro*, is somehow stabilized in whole Ehrlich-Lettre hyperdiploid ascites cells that are lacking in glucose. They also reported that, although K^+ caused no change in the properties of hexokinase

*This research was supported in part by Research Grant 10546 from the National Institutes of Health and by Grant DMB-8502211 from the National Science Foundation. Journal Paper No. J-12307 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Ia. 50011, Project 2575.

0006-291X/86 $1.50

II, it did serve to stabilize the enzyme against thermal inactivation and that the thermal stability of the hexokinase II was further increased by the addition of both K^+ and glucose (8). On the basis of these results, Rose and Warms (8) suggested that the changes in the K^+ and glucose levels that occur in the diabetic state could contribute to the decrease in hexokinase II in insulin-sensitive tissues. Conversely, it is reasonable to suppose that the increase in K^+ and glucose upon treatment with insulin could contribute to an increase in the stability of hexokinase II.

In light of these findings, we thought it would be useful to investigate the effect of insulin on the rate of hexokinase II degradation in the streptozotocin-diabetic rat. The results of these investigations are presented in this report.

EXPERIMENTAL

MATERIALS

White male Wistar rats were obtained from Hill Top Laboratories, Inc. L-[U-^{14}C]leucine (300Ci/mol) and L-[4,5-^{3}H]leucine (61Ci/mol) were purchased from Amersham. Protosol was obtained from New England Nuclear. Pansorbin (10% w/v Staphlococcus aureus cell suspension) was bought from Calbiochem. Lente insulin (Squibb) and Chemstrip bG (Boehringer Mannheim) were supplied by a local pharmacy. Insulin radioactive immune assay kit (RIA) was obtained from Radioassay Systems Laboratories, Inc. Distilled, deionized water was used in the preparation of all reagents. All other chemicals were of the highest purity commercially available.

METHODS

The diabetic state was induced in male rats, weighing 180-200 g, by intravenous injection of streptozotocin (75mg/kg body weight) following a 24-hour fast (9). Rats used as diabetic had a blood glucose level of greater than 400mg/100ml and immunoreactive insulin levels below 4μU/ml. The diabetic rats then received a daily intramuscular injection of 1.5 U Lente insulin/100 g body weight. This treatment was continued for 5 days, during which blood glucose levels returned to normal values (average insulin-treated = 112mg/100ml; average normal levels = 140mg/100ml), and immunoreactive insulin levels returned to near normal levels (average insulin-treated =31μU/ml; average normal = 53μU/ml).

Blood glucose was determined on a daily basis by using a chemstrip bG for glucose in whole blood and experimentally by using a hexokinase/glucose-6-P dehydrogenase coupled assay (10). The assay contained 20 mM HEPES buffer (pH 8.0), 10 mM ATP, 10 mM $MgCl_2$, and 0.4 mM NADP in a total volume of 1 ml .

Serum insulin levels were determined with an insulin RIA kit (11).

Hexokinase II was purified from rat skeletal muscle, and antibodies to the enzyme were produced as described elsewhere (4,6). Immunoprecipitation of hexokinase II was carried out as reported earlier (6). The radioactivity incorporated into cytosolic proteins was determined as indicated elsewhere (6).

MEASUREMENT OF ENZYME DEGRADATION

The fractional turnover rate of hexokinase II in normal and diabetic rat skeletal muscle was estimated by using a double-isotope method as described

by Arias et al. (12) and Glass and Doyle (13). At zero time (t = 0 hour), [^{14}C]leucine (13μCi/100 g of body weight) was injected. Additional injections of [^{3}H]leucine (26μCi/100 g of body weight) were administered 12, 24, and 36 hours later. After one hour exposure to the [^{3}H]-label, the rats were sacrificed, and procedures described elsewhere were followed to determine the rates of protein degradation (6).

From the estimated [^{3}H/^{14}C] ratios measured in the hexokinase II immunoprecipitate, the rate constant for degradation (K_d) was calculated according to the equation (14):

$$K_d\ (hr^{-1}) = \frac{2.303}{t-t_o} \times \log \left[{}^{3}H/_{14}C \right]_{t_o} \Big/ \left[{}^{3}H/_{14}C \right]_{t} \qquad (1)$$

The apparent half-life ($t_{1/2}$) of hexokinase II was calculated from the relationship:

$$t_{1/2} = \ln 2/K_d \qquad (2)$$

Statistical differences were analyzed by using student's t-test (15).

RESULTS AND DISCUSSION

Figure 1 illustrates the rates of degradation of hexokinase II in the normal, streptozotocin-diabetic, and insulin-treated diabetic rat skeletal

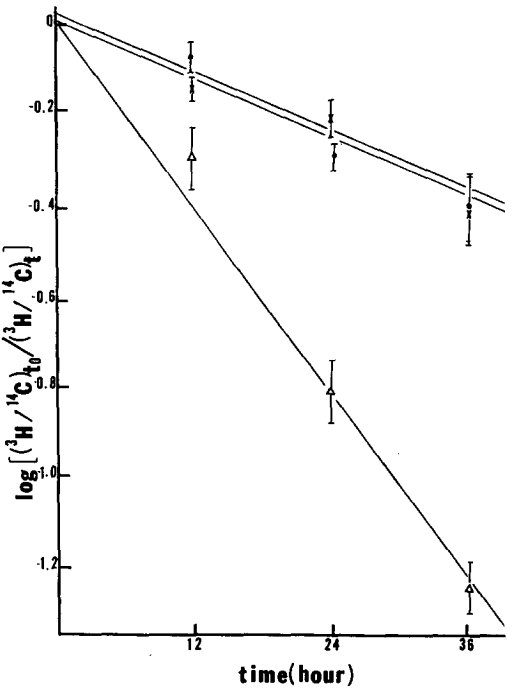

Figure 1. Plot of log $(^{3}H/^{14}C)_{t=o}/(^{3}H/^{14}C)_t$ versus time for hexokinase II in the skeletal muscle of the normal (x), diabetic (Δ), and diabetic insulin-treated (•) rats. Points are graphed as the mean ± standard deviation. Lines are least-squares computer fits to the data.

Table I

Protein degradation in diabetic insulin-treated rats*

Rats	Time (Hr)	No. of Rats	^{3}H dpm / mg muscle	Hexokinase II ^{14}C dpm / mg muscle	$^{3}H/^{14}C$	Cytosolic Protein $^{3}H/^{14}C$
Diabetic-insulin	0	4	1.12±0.42	0.84±0.15	1.51±0.29	3.18±1.08
	12	4	1.77±0.26	1.05±0.13	1.69±0.24	3.27±0.44
	24	5	1.64±0.16	0.65±0.01	2.55±0.03	3.36±0.98
	36	5	1.53±0.25	0.49±0.05	3.69±0.26	4.26±1.32

*$^{3}H/^{14}C$ ratios in immunoprecipitable hexokinase II and cytosolic protein in skeletal muscle of the diabetic insulin-treated rat. Points represent mean± standard deviation. See "Materials and Methods" for details.

muscle. From the data, it can be seen that, upon treatment of the diabetic rats with insulin, the rate of hexokinase II degradation decreases to normal levels. As previously reported (6), the rate constant for degradation, K_d, for hexokinase II is greater in the diabetic state, by a factor of 3, than in the normal animal. The K_d for hexokinase II in the insulin-treated rat was 3 times less than in the diabetic animal and equal to the rate in the normal animal. The incorporation of [^{14}C]leucine and [^{3}H]leucine into cytosolic proteins was also determined, and the results of these investigations can be found in Table I.

Figure 2 illustrates the rates of degradation of the cytosolic proteins in the normal, streptozotocin-diabetic, and diabetic insulin-treated rat skeletal muscle. It is clear that the differences in the rate of degradation between the normal and diabetic and the diabetic insulin-treated and the diabetic rat, are greater for hexokinase II (Figure 1) than the analogous differences for the cytosolic proteins (Figure 2). The apparent rate constants of degradation and the apparent protein half-lives are summarized in Table II.

To more clearly express the change in the apparent rate constants for degradation in the different physiological states, the [$^{3}H/^{14}C$] ratios found for hexokinase II were divided by the [$^{3}H/^{14}C$] ratios recorded for the average of the total cytosolic proteins. These corrected [$^{3}H/^{14}C$] ratios can then be

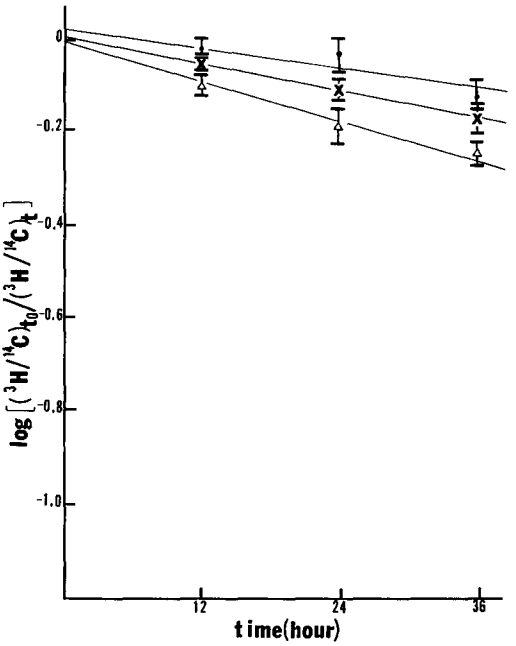

Figure 2. Plot of log $(^3H/^{14}C)_{t=0}/^3H/^{14}C)$ versus time for cytosolic proteins in the skeletal muscle of the normal (x), diabetic (Δ), and diabetic insulin-treated (•) rat. Points are graphed as the mean ± standard deviation. Lines are least-squares computer fits to the data.

plotted as shown in Figure 3. Again, the apparent rate constant of degradation, K_d, can be calculated from the slopes of the lines illustrated in Figure 3. The apparent rates of degradation and half-lives calculated from

Table II

Rate constants and half-lives of hexokinase II and cytosolic proteins in normal, diabetic, and diabetic insulin-treated rat skeletal muscle

Animal	hexokinase II		cytosolic proteins	
	$k_d(hr^{-1})^a$	t $(hr)^b$	$k_d(hr^{-1})^a$	t $(hr)^b$
Normal	0.025[c]	28[c]	0.012[c]	60[c]
Diabetic	0.078	9	0.015	43
Diabetic-insulin	0.025[c]	28	0.009	75[c]

[a] k_d = apparent rate constant of degradation.

[b] $t = \ln 2/k_d$.

[c] $P < 0.005$ versus diabetic.

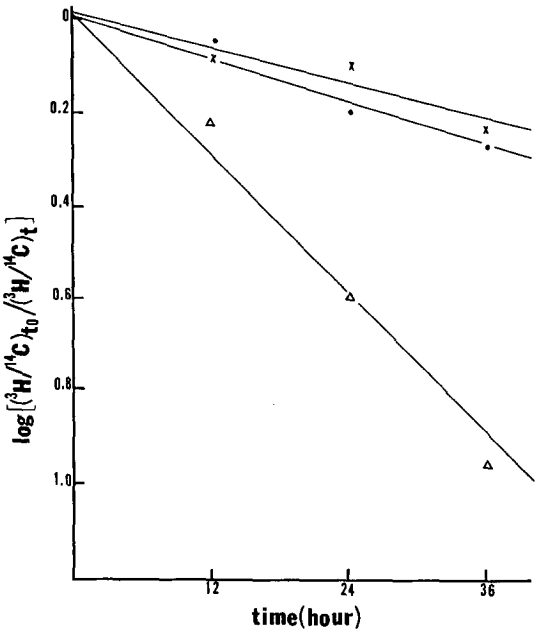

Figure 3. Degradation of hexokinase II relative to the average of the total cytosolic proteins in skeletal muscle of the normal (x), diabetic (Δ), and diabetic insulin-treated (•) rat.

these data are summarized in Table III. It can be seen that the apparent half-life of hexokinase II is approximately 2.3 times greater in the normal as compared with the diabetic rat. The apparent half-life of hexokinase II is about 2 times larger in the diabetic insulin-treated rat as compared with the diabetic animal. From these results, it is clear that insulin does decrease the rate of hexokinase degradation in the skeletal muscle of the diabetic

Table III

Degradation of hexokinase II relative to the average of the total cytosolic proteins

Animal	$k_d(hr^{-1})$	t (hr)
Normal	0.028	25[a]
Diabetic	0.064	11
Diabetic-insulin	0.032	22[a]

[a] $P < 0.005$ versus diabetic

rat; however, the cause of this restoration of the rate of hexokinase II degradation to normal values in the insulin-treated diabetic rat is not known.

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