Insulin Binding and Degradation by Muscles from Streptozotocin-diabetic Rats

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SUMMARY

Insulin degradation by muscle was examined in normal, streptozotocin-induced diabetic rats, and diabetic rats treated with insulin. Insulin degradation by the 100,000 × *g* supernatant fractions was identical in all three groups, but insulin metabolism by the intact epitrochlaris muscle was significantly increased in diabetic animals. Insulin treatment of the diabetic animals partially restored the activity toward normal. Specific binding of ^{125}I-insulin to the intact muscles was also increased in the diabetic animals. Streptozotocin diabetes, therefore, increased the binding and degradation of insulin by intact muscle but did not alter the insulin degradation by the total soluble intracellular degradative activity.

DIABETES *28*:746–748, August 1979.

The interaction of insulin with target tissues such as liver, fat, and muscle involves a number of different processes. The initial step is binding of the hormone to a specific receptor.[1] Subsequent to this, the hormone–receptor complex is "processed," an incompletely understood event which may result in internalization and/or degradation of the hormone.[2] Alterations in the binding step produced by diabetes have been well studied, particularly in fat and liver, but alterations in degrading activity are not as well established.[3,4] In particular, the metabolism of insulin by intact muscle has not been examined in detail.

We now report the effect of streptozotocin diabetes on insulin-degrading activity in both intact muscle and muscle homogenates and present data on insulin binding to the intact muscle.

METHODS

Male Holtzman rats (150–200 g) were used for all studies. The animals were injected with 65 mg/kg streptozotocin in 0.1 M citrate buffer, pH 4.5, or with citrate buffer alone. One week later the animals were killed by decapitation and the intact epitrochlaris muscles rapidly removed for studies with intact muscle[5,6] and the hind leg muscles removed for homogenization.

On the morning after the streptozotocin injections, one group of diabetic rats was anesthetized, and an osmotic minipump (Alza Corp.) designed to deliver 0.85 μl/h insulin was implanted subcutaneously in each animal's back. Each animal received 9.2 U regular insulin/day. At the time of death the plasma glucose (mean ± SEM) of the insulin-treated animals was 232 ± 31 mg/dl as compared with 553 ± 22 mg/dl for untreated diabetic rats and 99 ± 4 mg/dl for control animals ($P < 0.001$ for all groups).

The intact epitrochlaris muscles were immediately rinsed in ice-cold saline, weighed, and placed in Krebs-Ringer-Hepes (KRH) buffer, pH 7.4. Any muscles torn or cut during removal were discarded. The muscles were preincubated for 15 min at 37 °C in a shaking water bath and then transferred to vials containing ^{125}I-insulin (1×10^{-10} M) in KRH buffer (total volume 1 ml) and incubated 15 min at 37 °C.[6] In addition, in some experiments, ^{125}I-insulin was added to the media in which the muscles had been preincubated and the media incubated at 37 °C for an additional 15 min to determine the amount of degrading activity "leaked" from the muscles. At the end of the incubation, duplicate 100-μl aliquots were removed and added to vials containing 0.9-ml ice-cold buffer with 0.25% bovine serum albumin. One milliliter of 10% trichloroacetic (TCA) acid was added, and the percent degradation calculated from the increase in soluble radioactivity in the presence of muscle over control tubes incubated without muscle. One unit of activity is arbitrarily defined as that amount of enzyme that degrades 1% of the ^{125}I-insulin to TCA-soluble material under the defined conditions.

For the binding studies,[7] the muscles were incubated at 10 °C for 4 h in a shaking bath. One muscle from each animal was incubated with ^{125}I-insulin alone and the other muscle incubated with ^{125}I-insulin and 1×10^{-6} M unlabeled insulin for determination of nonspecific binding. At the end of the incubation the muscles were washed five times with ice-cold saline with 0.5% bovine serum albumin. The muscles were then counted in a Packard Autogamma spectrometer and the percent specific binding determined by the difference between the counts bound in the absence and in the pres-

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Received for publication 5 March 1979 and revision received 14 May 1979.

TABLE 1
Insulin-degrading activity of intact epitrochlaris muscles

	N	Units/mg wet wt	P	Units/mg protein	P
Control	10	0.297 ± 0.016		0.98 ± 0.06	
Diabetic rats	16	0.372 ± 0.031	<0.05	1.36 ± 0.10	<0.01
Treated diabetic rats	7	0.319 ± 0.27	NS*	1.11 ± 0.07†	NS

* Not significant.
† $P < 0.05$ compared with diabetic rats.

ence of unlabeled insulin. Nonspecific binding was approximately 25% of the total bound counts.

The hind leg muscles were homogenized in 0.33 M sucrose as previously described.[8] The homogenate was centrifuged at 100,000 × *g* for 60 min and the supernatant assayed for insulin-degrading activity by the TCA acid assay as described previously.[9] The supernatant was diluted so that in any assay not more than 15% of the insulin was degraded. The 100,000 × *g* fraction contains over 95% of the total insulin-degrading activity of the homogenate.[10]

^{125}I-insulin (SA 150–200 μCi/μg) was purchased from Immunonuclear Corp. and purified by passage over a DEAE cellulose column. Unlabeled insulin was a gift of Dr. R. Chance, Eli Lilly Co.

RESULTS AND DISCUSSION

Insulin degradation by the intact epitrochlaris muscle from normal and streptozotocin-diabetic rats is shown in Table 1. Muscles from diabetic animals had increased degrading activity as compared with normals, expressed either as activity per milligram wet weight or as activity per milligram protein. Administration of insulin to the diabetic animals decreased the degrading activity toward normal. Expressed as activity per milligram protein, the insulin-treated animals had significantly less activity than did the untreated diabetic animals.

There was no significant difference between control and diabetic muscles in the amount of degrading activity found in the preincubation media (data not shown), suggesting that the difference between control and diabetic muscle is not due to increased "leakiness" of the diabetic tissue. In addition, the weight of the control muscles (29.5 ± 1.6 mg) or the protein content (9.3 ± 0.2 mg) was not significantly different from the weight (28.2 ± 1.3 mg) or protein content (7.9 ± 0.3 mg) of the diabetic muscles.

Muscle homogenates from streptozotocin-diabetic animals did not have any change in insulin-degrading activity in the 100,000 × *g* supernatant fractions either with or without insulin treatment (Table 2).

TABLE 2
Insulin-degrading activity of supernatant fraction of muscle homogenates

	N	Units/mg protein	P
Control	11	181 ± 16	
Diabetic rats	14	177 ± 16	NS*
Treated diabetic rats	7	182 ± 23	NS*

* Not significant.

TABLE 3
Binding of ^{125}I-insulin to intact epitrochlaris muscles

	% Bound/mg wet wt	P	% Bound/mg protein	P
Control	0.0282 ± 0.0026		0.114 ± 0.011	
Diabetic rats	0.0394 ± 0.0036	<0.05	0.158 ± 0.013	<0.05

Table 3 shows that the specific binding of ^{125}I-insulin to the intact muscles was increased in the diabetic animals.

Insulin degradation by intact cells is apparently mediated through binding of insulin to specific receptors as the initial step. Insulin degradation by isolated liver cells is proportional to the amount of bound hormone,[11] and insulin bound to fat cell receptors is also readily degraded.[12] Because of this, the number of insulin receptors on the membrane may be a more important determining factor in the rate of insulin degradation by intact tissues than the amount of intracellular degrading enzymes. The total amount of insulin-degrading activity in cell homogenates is considerably greater than can be measured in intact cells.[13] In states of hyperinsulinemia,[14] resulting in a decreased number of receptors, insulin degradation by the liver is decreased in spite of an increase in total degrading activity.[15] In addition, insulin metabolism by muscle is impaired in uremic animals,[6,16] but the extent of impairment is far greater in intact muscles than in muscle homogenates.[6] Thus, the degradative activity of intact tissue may not always be accurately assessed by examining the total degradative activity of disrupted cells.

The data from the present study support this concept. Insulin degradation by the intact epitrochlaris muscle was increased in streptozotocin-diabetic animals, although the total insulin-degrading activity in the muscle homogenates was unchanged. Insulin binding by intact muscle was also increased in diabetic animals. Additional studies will be required before the binding and the degradation can be directly correlated, but these data suggest that binding and degradation may be related in muscle as in fat and liver.

ACKNOWLEDGMENTS

The excellent technical assistance of Martha Heinemann is gratefully acknowledged.

This study was supported in part by funds from the Veterans Administration, the National Institutes of Health Training Grant AM 07088, AM 18022, General Clinical Research Center Grant RR00211, and the Juvenile Diabetes Foundation. William C. Duckworth is the recipient of Research Career Development Award AM 00187 from the National Institutes of Health. Dan Gifford and Keith Runyan were recipients of Research Fellowships from the Memphis Mid-South Diabetes Affiliate.

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