Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting

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Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. Am. J. Physiol. 276 (Cell Physiol. 45): C1132–C1138, 1999.—The ubiquitin-proteasome proteolytic system is stimulated in conditions causing muscle atrophy. Signals initiating this response in these conditions are unknown, although glucocorticoids are required but insufficient to stimulate muscle proteolysis in starvation, acidosis, and sepsis. To identify signals that activate this system, we studied acutely diabetic rats that had metabolic acidosis and increased corticosterone production. Protein degradation was increased 52% (P < 0.05), and mRNA levels encoding ubiquitin-proteasome system components, including the ubiquitin-conjugating enzyme E2 has, were higher (transcription of the ubiquitin and proteasome subunit C3 genes in muscle was increased by nuclear run-off assay). In diabetic rats, prevention of acidemia by oral NaHCO₃ did not eliminate muscle proteolysis. Adrenalectomy blocked accelerated proteolysis and the rise in pathway mRNAs; both responses were restored by administration of a physiological dose of glucocorticoids to adrenalectomized, diabetic rats. Finally, treating diabetic rats with insulin for ≥24 h reversed muscle proteolysis and returned pathway mRNAs to control levels. Thus acidification is not necessary for these responses, but glucocorticoids and a low insulin level in tandem activate the ubiquitin-proteasome proteolytic system.

protein degradation; transcription; insulin; glucocorticoids

The principal effect of insulin on protein metabolism is to suppress protein degradation (9), and in insulin-dependent diabetic patients, insulin deprivation stimulates whole body protein degradation and amino acid oxidation (20, 21). Rats respond to insulin deprivation in vivo by increasing protein degradation (27), and insulin also suppresses protein degradation in cultured muscle cells (8, 10). These results suggest that a low insulin level, or possibly insulin resistance, could activate protein degradation in muscle with loss of muscle protein and decrease in lean body mass. In fact, we found that acute diabetes in rats produced by streptozotocin (STZ) injection results in muscle atrophy due to accelerated protein degradation by the ubiquitin-proteasome proteolytic pathway (22).

The ubiquitin-proteasome system is the major pathway accounting for the turnover of muscle protein, and it is activated in several catabolic conditions (18). Substrate proteins degraded by this system are first marked by conjugation to ubiquitin in ATP-dependent reactions. The ubiquitin-protein conjugates are then degraded by the 26S proteasome in a process that unfolds the protein, releases ubiquitin, and degrades the protein to small peptides and amino acids (6, 18). In acute diabetes, as in other catabolic conditions, activation of this pathway in muscle is associated with an increase in the content of mRNAs encoding components of the pathway (18, 22). On the basis of results from a nuclear run-off experiment, we determined that at least the higher level of ubiquitin mRNA in muscle of rats with chronic uremia or acute diabetes is the result of an increase in gene transcription (3, 22).

Even though the ubiquitin-proteasome system is responsible for degrading the bulk of protein in all cells, signals that activate this proteolytic pathway are unclear. Certain stimuli have been associated with activation of the ubiquitin-proteasome pathway in muscle. For example, in normal rats or rats with chronic renal failure, we found that acidification activates the ubiquitin-proteasome system (3, 17, 19). Another potential signal is an increase in glucocorticoids: pharmacological doses increase muscle proteolysis (12), and physiological levels are necessary but not sufficient for the catabolic responses in rats with metabolic acidosis, starvation, or sepsis (16, 23, 28, 32). Acidosis, increased glucocorticoid production, or a low level of insulin could function as signals activating muscle proteolysis in acute diabetes. We investigated which of these stimuli activate the ubiquitin-proteasome proteolytic system in muscle.

METHODS

Materials. STZ was purchased from Pfanstiehl Laboratories (Waukegan, IL), ZetaProbe GT membranes from Bio-Rad Laboratories (Hercules, CA), [³²P]dCTP and [³²P]CTP from Amersham (Arlington Heights, IL), TriReagent from Molecular Research Center (Cincinnati, OH), protamine-zinc-insulin (PZI) and protamine-zinc-anulin from Anpro Pharmaceutical (Arcadia, CA), Humulin R insulin from Eli Lilly (Indianapolis, IN), and Multistix 10 SG reagent strips from Miles (Elkhart, IN). All other chemicals or reagents were purchased from Sigma Chemical (St. Louis, MO). The proteasome inhibitor MG-132 was generously provided by ProScript (Cambridge, MA).

Rat model. After anesthesia, 125- to 150-g, male Sprague-Dawley rats (Charles River, MA) were given a tail vein injection of STZ (125 mg/kg prepared fresh in 0.1 M citrate buffer, pH 4.0) and pair fed a 23% protein diet with vehicle-injected, control rats, as described elsewhere (22). Rats were housed in individual cages for the duration of the experiment and studied 3 days (~72 h) after STZ injection. The only exception was when we investigated how rapidly insulin would reverse muscle proteolysis (see below). Urine was
collected during the 24-h period immediately before the experiments to measure corticosterone excretion to assess glucocorticoid production. We used this method because handling rats to obtain blood levels can acutely change the blood corticosterone level, and the daily excretion rate yields an estimate of the integrated, steady-state production rate (4, 13, 17).

To examine the influence of acidosis on muscle protein degradation, control and STZ-treated rats were given a solution of NaHCO3 by gavage, as described previously (24); other STZ-treated rats were given an equivalent amount of sodium as NaCl throughout the experiment. Pair-fed control rats were given NaHCO3 in a manner identical to STZ-treated rats.

To determine the role of glucocorticoids in the proteolytic response in muscle, 75-g rats underwent bilateral adrenalectomy (ADX) and were given 0.077 M NaCl to drink. After a 10-day recovery period, ADX rats were injected with STZ (125 mg/kg body wt) in the morning and were given the 23% protein diet and 10% glucose-0.077 M NaCl to drink ad libitum for 24 h. Subsequently, they were given 0.077 M NaCl to drink ad libitum. Another group of ADX-STZ rats was given dexamethasone (2 μg·100 g body wt −1·day −1·sc) in two equal injections starting on the day of STZ injection. Control ADX rats were treated in a similar fashion, except they did not receive STZ. ADX-control and ADX-STZ rats were pair fed to ADX-STZ rats receiving dexamethasone and studied 3 days (−72 h) after STZ injection.

To determine that the proteolytic response in muscles of STZ-treated rats is due to insulin insufficiency, STZ-treated rats were given a daily injection of the long-acting bovine PZI (8 U/100 g body wt sc) beginning on the morning of the STZ injection (day 1) and on the subsequent morning (day 2). Rats were fasted the night before muscles were isolated for measurements of protein degradation and levels of mRNAs encoding components of the ubiquitin-proteasome pathway (day 3).

To examine whether the proteolytic response in diabetic rats could be reversed by insulin, rats were injected with STZ and pair fed with control, sham-injected rats. The diabetic rats were not treated with insulin for the initial 3 days, and on the morning of day 3 they were randomly divided into two groups: 1) STZ-treated rats that received the shorter-acting Humulin R insulin (2.5 U/100 g body wt) and the longer-acting bovine protamine-zinc- insulin (0.5 U/100 g body wt) to have a sustained action of insulin and 2) STZ-treated rats that did not receive insulin. Pair feeding was continued during the day; after an overnight fast, muscles were isolated and studied.

Measurement of muscle protein degradation. The mixed-fiber epitrochlearis muscle was studied because it exhibits rates of protein turnover in the presence or absence of insulin that are similar to those measured in the bulk of muscle in adult rats (5). Epitrochlearis muscles were dissected from diabetic and control rats and preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate media containing 10 mM glucose and 0.5 mM cycloheximide and equilibrated with 95% O2-5% CO2 (pH 7.4) (3, 19, 22). The muscles were then placed in fresh media, regassed, and incubated for 2 h at 37°C. At the end of the incubation period, TCA (final concentration 10%) was added to precipitate proteins. Free tyrosine in the media was measured to calculate the rate of protein degradation, because we have found that tyrosine does not accumulate in the intracellular space in muscles of diabetic or control rats treated with inhibitors of proteolytic pathways (22). To evaluate changes in the activity of specific proteolytic pathways, inhibitors were added as described in earlier studies (3, 19, 22).

Northern blot hybridizations and nuclear run-off assay. RNA was isolated from the gastrocnemius muscles using TriReagent and separated in a formaldehyde-agarose gel by electrophoresis before transfer to a ZetaProbe GT membrane. Hybridizations were performed as described previously (22). The gastrocnemius muscle was studied, because it is a mixed-fiber muscle and changes in protein turnover reflect those occurring in the epitrochlearis muscle (5), and levels of mRNAs encoding components of the ubiquitin-proteasome pathway are coordinated with activation of this pathway in rats with acidosis, chronic renal failure, or starvation (3, 19, 22, 23).

To perform nuclear run-off assays, nuclei were isolated from hindquarter muscles from each rat. Transcription measurements were as described previously (3, 22).

Statistical analysis. Values are mean ± SE. Results were analyzed by using the Student’s paired t-test when results from two experimental groups were compared or by using ANOVA when data from more than two groups were studied. For data analyzed by ANOVA, pairwise comparisons were made by the Student-Newman-Keuls test. P < 0.05 was considered significant.

RESULTS

Acidosis and muscle proteolysis. Previously, we found that metabolic acidosis in otherwise normal rats stimulates the ubiquitin-proteasome pathway in muscle (19). Because STZ-treated rats develop acidosis (22), acidification could be the stimulus accelerating muscle protein degradation. To examine this possibility, rats were given oral NaHCO3 for 3 days (−72 h) after the STZ injection to prevent acidaemia. The serum HCO3 concentration was not different between pair-fed, control, and STZ-treated rats given NaHCO3 but was lower in STZ-treated rats given NaCl. Giving acutely diabetic rats NaHCO3 did not correct their hyperglycaemia (Table 1).

Protein degradation rates in muscles from STZ-treated or STZ-HCO3 rats were greater than the rate measured in control rat muscles (Fig. 1). When inhibitors of lysosomal and calcium-dependent proteolysis were added, protein degradation rates measured in muscles of STZ-treated rats or STZ-HCO3 rats were greater than the rates measured in muscles of control rats (Fig. 1). These results indicate that the ubiquitin-proteasome system must be responsible for the accelerated protein degradation.

When acutely diabetic rats were given NaHCO3, the level of ubiquitin mRNA in mixed-fiber gastrocnemius muscles was reduced by 10.2 ± 0.3-fold from control (246 ± 24.6 pmol/mg muscle protein) to 24.6 ± 2.4 pmol/mg muscle protein.

Table 1. Serum HCO3 and blood glucose values for control rats, acutely diabetic rats, and acutely diabetic rats given NaHCO3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HCO3, mM</th>
<th>Glucose, mg/dl</th>
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<tbody>
<tr>
<td>Control</td>
<td>24.1 ± 1.4</td>
<td>106 ± 6</td>
</tr>
<tr>
<td>STZ-HCO3</td>
<td>26.0 ± 0.5</td>
<td>281 ± 14*</td>
</tr>
<tr>
<td>STZ</td>
<td>17.4 ± 3.7*</td>
<td>320 ± 8*</td>
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Values are means ± SE. Streptozotocin (STZ)-treated rats were given oral NaCl (STZ) or NaHCO3 (STZ-HCO3) for 3 days before muscles were isolated, and arterial blood was sampled. *Significantly different from control (P < 0.05).
muscle was as high as in muscle of untreated, acutely diabetic rats and was 147% (P < 0.05) greater than the level in muscles of control rats (Fig. 2). Similarly, the levels of mRNAs encoding the C3 and C9 α-type proteasome subunits as well as the C5 β-type subunit were higher in muscle of diabetic rats and diabetic rats given NaHCO3 than in control rat muscles (Fig. 2). The levels of the 1.2-kb mRNA of the E214k ubiquitin-conjugating enzyme in STZ-treated or STZ-HCO3 rat muscles were also higher than in control rats. The level of the 1.8-kb E214k was higher in diabetic rats given NaHCO3 (Fig. 2). The reason for the increased level of the 1.8-kb mRNA in muscle of STZ-treated rats given NaHCO3 is unknown. These data, together with the muscle proteolysis measurements, indicate that activation of the ubiquitin-proteasome pathway by acute diabetes is not dependent on acidemia.

Glucocorticoids and muscle proteolysis. Corticosterone excretion was significantly (P < 0.001) less in pair-fed, control rats than in diabetic rats (Fig. 3), indicating that glucocorticoid production is increased by acute diabetes as it is in patients (25, 26). To evaluate the role of glucocorticoids in the proteolytic response to acute diabetes, we studied ADX rats. Results from ADX-control rats were compared with rates measured in muscle of ADX rats given STZ with (ADX-STZ-GC) or without (ADX-STZ) dexamethasone at a dose that approximates physiological glucocorticoid levels (16, 17). After an overnight fast, blood glucose levels in ADX-control, ADX-STZ, and ADX-STZ-GC rats were 51 ± 6, 160 ± 45, and 236 ± 26 mg/dl, respectively. Protein degradation was higher in muscles of ADX-STZ-GC rats replaced with glucocorticoids than in muscles of ADX-control or ADX-STZ rats that were not treated with glucocorticoids (Fig. 4). When lysosomal and calcium-dependent proteolysis inhibitors were present in the incubation media, proteolysis remained higher in muscles of ADX-STZ-GC rats than in ADX-STZ or ADX-control rats (Fig. 4).

Ubiquitin mRNA in muscles of ADX-control and diabetic ADX rats did not differ and were lower (P < 0.05) than in muscles of diabetic ADX rats given glucocorticoids (Fig. 5). Similarly, levels of mRNAs

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**Fig. 1.** Acidosis is not a stimulus of ubiquitin-proteasome-dependent protein degradation in muscles of acutely diabetic rats. Protein degradation was measured in isolated epitrochlearis muscles of pair-fed, control rats given oral NaHCO3 (CTL), acutely diabetic rats given oral NaHCO3 [streptozotocin (STZ) + HCO3], or diabetic rats given oral NaCl (STZ). Studies were begun 3 days after STZ injection. One muscle from each rat was incubated without proteolytic inhibitors (solid bars); the contralateral muscle was incubated in the presence of lysosomal and calcium-dependent proteolytic inhibitors (overlapping open bars). *P < 0.05 vs. control muscle protein degradation under same incubation conditions (n = 6 for each group).

**Fig. 2.** Acidosis is not a stimulus that increases muscle levels of ubiquitin (Ub)-proteasome system mRNAs in acute diabetes. RNA was isolated from gastrocnemius muscles of pair-fed control rats given oral NaHCO3 (CTL), acutely diabetic rats given oral NaHCO3 (STZ-HCO3), or diabetic rats given oral NaCl (STZ). Studies were begun 3 days after STZ injection. A: Northern blots were hybridized with cDNAs for chicken ubiquitin, rat E214k ubiquitin-conjugating enzyme, and rat C3, C5, and C9 proteasome subunits; corresponding 18S and 28S rRNA bands are also shown. B: RNA band intensities were quantified, and results from 4 rats were assessed. Values are normalized using 28S rRNA values and are expressed as percentage (mean ± SE) of mean control rat value. Solid bars, diabetic rats given NaHCO3; open bars, diabetic rats given NaCl. *P < 0.05 vs. control.

**Fig. 3.** Corticosterone production is increased by acute diabetes. Urine was collected for 24 h, and total daily excretion of corticosterone was measured. Experimental collections were performed between days 2 and 3 in control (CTL) or diabetic rats (STZ) 3 days after STZ injection. Excretion rates for individual rats are shown. Mean (+) value in 8 STZ rats was higher (P < 0.001) than in 8 control rats.
encoding other pathway components were significantly lower (P < 0.05) in muscle of ADX-control and ADX-STZ rats than ADX-STZ-GC rats (Fig. 5).

Insulin and muscle proteolysis. We gave STZ rats daily doses of long-acting PZI beginning on the morning after the STZ injections (days 1 and 2). After an overnight fast and ~24 h after the last insulin injection, blood glucose concentrations in control and diabetic rats on day 3 were 116 ± 8 and 266 ± 49 mg/dl, respectively (P < 0.05 vs. control); blood glucose in diabetic rats given insulin was 330 ± 10 mg/dl (P < 0.05 vs. control). Corticosterone excretion during the 24 h after the last insulin injection and before muscles were isolated to measure protein degradation was 2.13 ± 0.20 µg/kg body wt in control rats, 3.18 ± 1.09 µg/kg body wt in diabetic rats receiving insulin (not significant vs. control rats), and 9.79 ± 3.36 µg/kg body wt in diabetic rats treated with insulin. Insulin prevented the accelerated rate of proteolysis in muscle. We attribute this result to suppression of proteolysis by the ubiquitin-proteasome system for two reasons: 1) protein degradation remained elevated in muscle of acutely diabetic rats, even though lysosomal and calcium-activated proteases were blocked (Fig. 6), and 2) when the proteasome inhibitor MG-132 was added, protein degradation in muscles from acutely diabetic rats was not different from the rate measured in muscle of control rats or diabetic rats receiving insulin (Fig. 6).

mRNA levels were higher (P < 0.05) in muscles of diabetic rats than in muscles of control, pair-fed rats, but when diabetic rats were given insulin for 3 days the levels of these mRNAs were not different from those measured in muscles of control rats (Fig. 7).
We also examined whether insulin would reverse the increase in muscle protein degradation and reduce the higher levels of mRNAs in muscle of diabetic rats. On day 4 after STZ injection the diabetic rats were arbitrarily divided into two groups that were pair fed: 1) diabetic rats that received insulin and 2) diabetic rats that did not receive insulin. Insulin treatment for 12 h did not give a consistent pattern of changes in muscle protein degradation. Insulin treatment for 24 h led to a blood glucose of 138 ± 6 mg/dl vs. 132 ± 16 mg/dl in control rats and 247 ± 14 mg/dl in acutely diabetic rats. The basal rates of protein degradation in muscles of control and acutely diabetic rats given insulin for 24 h were not different statistically (152.8 ± 5.8 vs. 156.1 ± 11.5 ng tyrosine·g⁻¹·h⁻¹, respectively). Both values were lower than the rate measured in muscle of diabetic rats (261.1 ± 11.6 ng tyrosine·g⁻¹·h⁻¹, P < 0.05). Ubiquitin and proteasome C3 subunit mRNA values in muscles of diabetic rats given insulin were also reduced to control levels (data not shown).

Proteasome subunit gene transcription. To evaluate whether the higher level of proteasome subunit mRNAs is related to increased gene transcription, nuclei were isolated from muscles of control and STZ-treated rats, and run-off assays were performed. A representative result (Fig. 8) indicates that transcription of the genes encoding the C3 proteasome subunit as well as ubiquitin is increased in muscles of STZ rats compared with control rats. In three pairs of rats with acute diabetes and their pair-fed controls, diabetes was associated with increased transcription of the C3 proteasome subunit and ubiquitin genes of 78 ± 12 and 58 ± 10%, respectively (P < 0.05 vs. pair-fed control rats). Transcription of the glyceraldehyde 3-phosphate dehydrogenase gene was unchanged by diabetes.

DISCUSSION

In adult humans the principal effect of insulin on protein metabolism is suppression of protein degradation (9, 15). This also appears to be true in rats, because we found that an acute decrease in insulin production causes substantial loss of body weight, reduced muscle, liver, and adipose tissue mass, and accelerated muscle protein catabolism via the ubiquitin-proteasome system (22). Potential signals initiating this proteolytic response could be metabolic acidosis and/or an increase in glucocorticoids, since metabolic acidosis induced by feeding NH₄Cl increases the steady-state production of glucocorticoids and stimulates muscle protein degradation, whereas high doses of glucocorticoids stimulate muscle protein degradation, at least transiently (1, 12). Finally, the proteolytic response could be due to a decrease in insulin level, which accelerates protein degradation, because the normal, insulin-related suppression of muscle protein catabolism is diminished (5).

We excluded metabolic acidosis as a prominent stimulus of muscle protein degradation caused by a low insulin level. When we gave NaHCO₃ to acutely diabetic rats, the accelerated rate of muscle protein degradation was unchanged in muscle, even though serum bicarbonate was normal. Likewise, preventing acidosis did not attenuate the rise in levels of mRNAs encoding components of the ubiquitin-proteasome pathway. Thus the acidosis of acute diabetes does not activate the
proteolytic system in muscle, although acidification will lower the pH in cultured muscle cells and stimulate protein degradation (8, 11). This was unexpected in light of our finding that correction of acidosis in chronically uremic rats blocks the increase in muscle proteolysis and the rise in ubiquitin and proteasome component mRNAs (3). The mechanism for activation of this system by acidemia is complicated, however, because we found that induction of metabolic acidosis in normal rats by feeding NH₄Cl lowers the pH in rat muscle (measured by NMR) by only a small amount, whereas the acidosis of chronic renal failure does not change muscle pH or the recovery of muscle pH after intracellular acidification resulting from muscle contraction (2). It is difficult to implicate changes in muscle pH as a primary signal activating the ubiquitin-proteasome system in muscle.

In normal rats the proteolytic response to acidification in muscle requires glucocorticoids (16, 17, 23). The present results demonstrate that glucocorticoids are also required for activation of the ubiquitin-proteasome pathway in acutely diabetic rats. First, urinary corticosterone excretion, a measure of the integrated rate of glucocorticoid production, is high in these rats (17). Second, ADX prevented the accelerated protein degradation in muscles of acutely diabetic rats, and the proteolytic response was restored when the ADX diabetic rats were given dexamethasone at a dose that yields physiological replacement levels. Moreover, the pattern of changes in the levels of mRNAs of the ubiquitin-proteasome pathway in muscle was consistent with the changes in muscle proteolysis (23). We did not evaluate the proteolytic or mRNA responses in normal (nondiabetic) ADX rats given the same amount of dexamethasone, because we have found that this dose of dexamethasone or one slightly higher does not increase muscle proteolysis or the levels of ubiquitin-proteasome pathway mRNAs (16, 17, 23). Thus the results we obtained in control and ADX diabetic rats are consistent with the conclusion that two stimuli, i.e., glucocorticoids and a low insulin level, are required to stimulate the ubiquitin-proteasome pathway.

The critical role of insulin in controlling muscle proteolysis was demonstrated by giving insulin to rats after STZ administration. Even though blood glucose was not corrected, insulin administration prevented the increase in muscle proteolysis and the rise in ubiquitin-proteasome pathway component mRNAs. These results suggest that the beneficial influence of insulin is independent of the blood glucose. Moreover, even when the ubiquitin-proteasome system is activated by acute diabetes, accelerated muscle proteolysis was reduced to a normal level after only 24 h of insulin. Likewise, insulin reduced the levels of mRNAs encoding components of the ubiquitin-proteasome system in muscle to levels measured in control rats. On the other hand, insulin did suppress glucocorticoid production. Again, these results point to a dual role of low insulin plus glucocorticoids as the signal activating the ubiquitin-proteasome pathway in muscle.

Interestingly, the rate of muscle proteolysis and levels of ubiquitin-proteasome pathway mRNAs were regulated in a coordinated fashion. In studies of ADX rats with diabetes or diabetic rats given insulin, a change in protein degradation was accompanied by a parallel change in mRNA levels. Thus we could not determine whether there is interdependence between the two types of responses, as occurs in septic rats, despite attempts to shorten the duration of time for exposure to insulin (29).

The E2₁₄k ubiquitin-carrier enzyme is encoded by two species of mRNA, 1.8- and 1.2-kb mRNA. These mRNAs differ in the length of their 3'-untranslated regions (3'-UTR), but their translation products are identical (30). It is not known whether these mRNAs are translated with equal efficiency. Notably, Wing and Bedard (31) found that insulin-like growth factor 1 or insulin will increase the rate of the 1.2-kb mRNA degradation but not of the 1.8-kb species and concluded that the additional 3'-UTR in the 1.8-kb E₂₁₄k mRNA confers stability (31). This pattern of regulation of the E₂₁₄k mRNAs is interesting, because we found that insulinopenia increases the 1.2- but not the 1.8-kb form. Our finding is consistent with activation of at least one ubiquitin-carrier protein, E₂₁₄k, and an increase in ubiquitin-conjugating activity in muscle (unpublished observations). Taken together, these studies make it tempting to speculate that one mechanism leading to suppression of protein degradation by insulin could involve reduced activity of one or more ubiquitin-carrier enzymes. We do not know why the 1.8-kb E₂₁₄k mRNA was increased in muscles of diabetic rats given bicarbonate inasmuch as this response was not found in any other group of diabetic rats. Possibly, the increase in this mRNA was in response to bicarbonate directly or their mild alkalosis (Table 1) rather than hyperglycemia or insulinopenia.

It is notable that the proteolytic response to acute diabetes and other catabolic conditions (e.g., acidosis and sepsis) not only involves glucocorticoids (17, 23, 28) but often is also associated with reduced or impaired responses to insulin [e.g., uremia, sepsis, and acidosis (7, 14, 17)], whereas starvation is associated with reduced insulin levels. Thus impaired insulin action and/or relative insulin deficiency could be a common factor activating protein degradation in these conditions, since the present results show that a low insulin level is a signal activating the ubiquitin-proteasome system.

In summary, muscle wasting in acute diabetes results from activation of the ubiquitin-proteasome proteolytic pathway by a mechanism that requires glucocorticoids. We have excluded a high blood glucose concentration or acidification as independent mediators activating muscle proteolysis in diabetes. It is tempting to speculate that the insulin signal transduction pathway acts to suppress the ubiquitin-proteasome system. In this case, insulin resistance in other conditions (e.g., sepsis, burn injury, and metabolic acidosis) would contribute to muscle atrophy, especially if glucocorticoid production were high.
REFERENCES


