Systemic Response to Thermal Injury in Rats

Accelerated Protein Degradation and Altered Glucose Utilization in Muscle

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Abstract. Negative nitrogen balance and increased oxygen consumption after thermal injury in humans and experimental animals is related to the extent of the burn. To determine whether defective muscle metabolism is restricted to the region of injury, we studied protein and glucose metabolism in forelimb muscles of rats 48 h after a scalding injury of their hindquarters. This injury increased muscle protein degradation (PD) from 140±5 to 225±5 nmol tyrosine/g per h, but did not alter protein synthesis. Muscle lactate release was increased >70%, even though plasma catecholamines and muscle cyclic AMP were not increased. Insulin dose-response studies revealed that the burn decreased the responsiveness of muscle glycogen synthesis to insulin but did not alter its sensitivity to insulin. Rates of net glycolysis and glucose oxidation were increased and substrate cycling of fructose-6-phosphate was decreased at all levels of insulin.

The burn-induced increase in protein and glucose catabolism was not mediated by adrenal hormones, since they persisted despite adrenalectomy. Muscle PGE_2 production was not increased by the burn and inhibition of prostaglandin synthesis by indomethacin did not inhibit proteolysis. The increase in PD required lysosomal proteolysis, since inhibition of cathepsin B with EP475 reduced PD. Insulin reduced PD 20% and the effects of EP475 and insulin were additive, reducing PD 41%. An inhibitor of muscle PD, α-ketoisocaproate, reduced burn-induced proteolysis 28% and lactate release 56%. The rate of PD in muscle of burned and unburned rats was correlated with the percentage of glucose uptake that was directed into lactate production ($r = +0.82$, $P < 0.01$). Thus, a major thermal injury causes hypercatabolism of protein and glucose in muscle that is distant from the injury, and these responses may be linked to a single metabolic defect.

Portions of this work were presented at the Annual Meeting of the American Society for Clinical Investigation, Washington, D. C., 1983.

Drs. Clark and Kelly are recipients of National Research Awards AM06660 and HL01368. Dr. Mitch is the recipient of a Research Career Development Award (AM00750) from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health. Address reprint requests to Dr. Mitch.

Received for publication 21 November 1983 and in revised form 7 June 1984.

J. Clin. Invest.

0021-9738/84/09/0888/10 $1.00
Volume 74, September 1984, 888–897

Introduction

In humans, thermal injury increases net urea nitrogen production and loss of lean body mass (1, 2). This suggests that net protein degradation (PD)[1] increases in muscle, since muscle is the most likely source of nitrogen that is required for excessive urea production. Thermal injury also is associated with increased lactate production and glucose recycling, which suggests that glucose metabolism in muscle is abnormal (3–6). Odessey and Parr (7) have found that in rats, there is increased PD in muscle underlying a burn, and Nelson and Turinsky (8, 9) reported that insulin-mediated glucose utilization is abnormal in muscle underlying a burn. Both glucose and protein metabolism were normal in muscles that were distant from the thermal injury. However, in these experimental models, the burn was restricted to a single hindlimb and may not have reproduced the hypercatabolic state that is characteristic of a major thermal injury (10, 11). In support of this contention, an increase in oxygen consumption by burned rats was not detected until the extent of the injury exceeded 50% of body surface area (12).

After a major burn, plasma levels of potentially catabolic hormones, including catecholamines, glucagon, and corticosteroids, increase (3, 5, 6, 11). In addition, a factor that was isolated from the plasma of traumatized patients has been reported to accelerate proteolysis in incubated muscles from

1. *Abbreviations used in this paper:* ADX, adrenalectomy, GO, glucose oxidation; GS, glycogen synthesis; αKIC, αketoisocaproate; PD, protein degradation; PGE_2, prostaglandin E_2; PS, protein synthesis; SC, substrate cycling.

normal rats (13). Therefore, it is possible that muscles which are distant from the burned area, as well as those underlying the burn, would participate in the catabolic response to a major thermal injury.

To study the consequences of a major thermal injury on muscle metabolism in rats, we measured in vivo the urea nitrogen appearance rate, and in vitro the rates of protein synthesis (PS) and degradation in muscle distant from the injury. We also studied the effects of insulin on muscle glucose utilization and compared the abnormalities uncovered with alterations in net protein degradation. Since we found accelerated proteolysis, we investigated the role of adrenal hormones and muscle prostaglandin synthesis and whether the increase in protein degradation was mediated by lysosomal proteolytic pathways.

Methods

Male Sprague-Dawley rats that weighed 180–220 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were maintained on a 12 h light-12 h dark cycle and allowed free access to water and RMH 1000 rat chow (Agway Country Foods, Agway Inc., Syracuse, NY) for at least 3 d before being studied. The rats were anesthetized with 5 mg/100 g body weight of sodium pentobarbital and 5 ml/100 g body weight of 150 mM saline were injected into the peritoneal cavity to protect the viscera from thermal injury. Thermal injury was induced by immersing the hindquarter to the mid-abdominal level in 90°C water for 3 s (14). This procedure produces an anesthetizing (7), full-thickness burn of 45–50% of body surface area. This technique was developed in accordance with the Guide for the Care and Use of Laboratory Animals (15) and in consultation with veterinary physicians. Control rats were treated similarly, except that they were immersed in 37°C water.

After immersion, rats were housed in individual, wire-bottomed cages in order to collect urine and prevent coprophagia. They were given free access to 75 mM saline, but food was withheld. After 44 to 48 h, the rats were anesthetized again and the forelimb, epitrochlearis muscles removed, weighed, and placed into flasks that contained 3 ml of Krebs-Henseleit bicarbonate buffer, 10 mM glucose, 0.5 mM phenylalanine, 0.2% fatty acid and globulin-free bovine serum albumin, and other additions as indicated. Flasks were gassed for 3 min with 95% O_2-5% CO_2, placed into a rotating incubator (60 cycles/min), and incubated for 30 min at 37°C. Muscles were then removed, blotted, transferred to flasks that contained fresh media, regassed, and incubated for a subsequent 2-h test period.

Protein synthesis was calculated by measuring the rate of incorporation of U-^{14}C-phenylalanine into muscle protein and dividing it by the specific radioactivity (0.1 mCi/mmol) of phenylalanine in the media (16, 17). PD was determined in separate experiments by measuring the rate of release of tyrosine into the media in the presence of 0.5 mM cycloheximide. Previously, we and others have found that tyrosine accumulation in the intracellular space was insignificant during a 2-h incubation (16, 18).

Muscle glucose metabolism was assessed in separate experiments by incubating with U-^{14}C-glucose (5 μCi/mmol) and 5-^{3}H-glucose (0.1 mCi/mmol) simultaneously. Following the experimental period, the stoppered flask was cooled on ice and 2 ml of media was withdrawn and injected into another stoppered flask that contained 0.5 ml of 50% TCA and a suspended center well that contained 0.2 ml phenethylamine. This flask was incubated for an additional hour. The phenethylamine was then removed and the $^{14}CO_2$ content was determined by scintillation counting with correction for quenching, using an external standard.

After removing 2 ml of media, the muscle was blotted and dissolved in 30% KOH at 100°C. Muscle glycogen was isolated by extraction into 85% ethanol; recovery of glycogen was 91±2%. The ^{14}C and ^{3}H content of glycogen was determined by liquid scintillation spectrometry, and glycogen concentration was measured spectrophotometrically (19). An additional 400 μl of media were added to 200 μl of 10% PCA and assayed for lactate concentration using lactate dehydrogenase. Tritiated water that was formed from fructose-1,6-diphosphate by the aldolase and triose phosphate isomerase reactions was measured using techniques described by Ashcroft et al. (20) and Zawalich et al. (21). Recovery of $^{3}H_2O$ by this technique during incubation for 18 h at 37°C was 73±2%.

The rate of net glycolysis was calculated as the difference between the rate of $^{3}H_2O$ formed (tritiated water) and the rate of fructose-6-phosphate formation from fructose-1,6-diphosphate, and the substrate cycling (Sc) estimate as detailed by Clark et al. (22) and Newsholme and Crabtree (23). The ratio of ^{3}H to ^{14}C in glycogen, instead of that in glucose-6-phosphate, was used to calculate Sc based on the results of Katz et al. (24) and Newsholme and Crabtree (23). If in muscle the release of ^{3}H from fructose-1,6-diphosphate were not complete as it is in other tissues (25), the rates of Sc and glycolysis would be underestimated. To examine this possibility, we compared this radiochemical measurement of glycolysis with that calculated as the sum of one-half of lactate release plus glucose oxidation (GO). There was no statistical difference between these two methods of measuring glycolysis. The rate of glycogen synthesis (GS) was calculated as the rate of ^{14}C-glucose incorporated into glycogen divided by the specific radioactivity of glucose in the media (26). GO was calculated as the rate of $^{14}CO_2$ evolved divided by the specific radioactivity of glucose in the media. In calculating GO and GS, we have assumed that the specific radioactivity of ^{14}C-glucose and glucose-6-phosphate are equal and remain constant, since lactate conversion to glycogen is minimal (27) at the concentrations of lactate (0.1–0.3 mM) found in our experiments. Glucose uptake (total glucose utilization) was calculated as the sum of GS and glycolysis which was measured during experiments with 5-^{3}H-glucose (26). Glucose transport was examined in separate studies by preincubating muscles in glucose-free media with or without 10 mU/ml insulin. The muscles were then incubated for 30 min with different concentrations of glucose and tracer amounts of either 2-deoxy-U-^{14}-glucose (0.2 μM; 10 μCi/mmol glucose) or 2-^{3}H-glucose (0.1 mCi/mmol) with and without insulin. The rate of accumulation of 2-deoxy-glucose in the intracellular space of muscle and the rate of $^{3}H_2O$ that was formed from 2-^{3}H-glucose (28) were measured in order to calculate glucose transport using the ratio of 2-deoxy-U-^{14}C-glucose/glucose and the specific radioactivity of 2-^{3}H-glucose in the media (26, 28, 29).

Fatty acid metabolism was studied by incubating muscles for a 2-h experimental period with 0.5 mM oleate and 4 g/dl albumin that contained 1-^{14}C-oleate (0.4 mCi/mmol). The rate of oxidation was calculated by dividing the rate of $^{14}CO_2$ evolved by the specific radioactivity of oleate in the media (30).

Tyrosine, phenylalanine, and urea were assayed fluorometrically (31–34). Glucose was measured using a Beckman Glucose II analyzer (Beckman Instruments, Inc., Palo Alto, CA), which we have found to have a coefficient of variation of 1.5% with glucose measured enzymatically (17). Urea nitrogen appearance was calculated as the algebraic sum of urea nitrogen excretion, and the change in the urea nitrogen

pool which was measured as the product of plasma urea nitrogen and the volume of distribution of ^{14}C-urea (35). In burned rats, the urea space was 51.5±1.6% body weight; it was 55.4±1.1% body weight in unburned control rats. Prostaglandin E_2 (PGE_2) production by incubated muscle was estimated by radioimmunoassay of PGE_2 that was released into the media (36, 37). Lysosomal cathepsin B activity in homogenates of epitrochlearis muscles was measured by monitoring the hydrolysis of the substrate, carbobenoxy-alanyl-arginyl-arginyl-4-methoxy-*p*-naphthylamine (CBZ-Ala-Arg-Arg-MNA) fluorometrically (38). A 10% (wt/vol) muscle homogenate in 0.15 M KCl (pH 5.0) was frozen at −70°C to disrupt lysosomes and a 25-μl aliquot was incubated at 37°C for 1 h with the substrate in 0.2 mM Na_2HPO_4, 0.2 mM citric acid buffer (pH 6.0) that contained 2 mM dithiothreitol, and 8 mM EDTA. Plasma glucagon was measured by radioimmunoassay using the 30,000 glucagon-specific antibody of Unger (39). Plasma catecholamine levels were measured radioenzymatically (40). The protein content of muscle was measured by the biuret method; the cyclic AMP content of muscle that was homogenized in 0.1 N HCl was measured by radioimmunoassay (41).

The effects of adrenal hormones on muscle metabolism were assessed after adrenalectomy using hormone replacement schedules as indicated. Adrenalectomy was performed through a dorsal incision 24 h before the thermal injury. Subsequently, these rats and their sham-operated controls were given 150 mM saline to drink.

Reagent grade chemicals were obtained from Sigma Chemical Co., St. Louis, MO; U-^{14}-C-phenylalanine was obtained from Schwartz/Mann Div., Becton-Dickinson & Co., Orangeburg, NY; other radiochemicals were obtained from New England Nuclear, Boston, MA. The fluorometric substrate, CBZ-Ala-Arg-Arg-MNA, was purchased from Enzyme Systems Products (Livermore, CA). The lysosomal thiol protease inhibitor, EP-475, was generously provided by Dr. A. L. Goldberg, Harvard Medical School, Boston, MA. Glucagon and porcine insulin were obtained from Eli Lilly & Co., Indianapolis, IN.

Results are presented as mean±SEM. A paired *t* test was used to assess the effects of an added hormone or test compound in incubations of paired muscles from individual rats. An unpaired *t* test was used to compare results that were obtained in different treatment groups. Results were considered significant at $P < 0.05$.

Results

Survival of rats after thermal injury to the hindquarter exceeded 97%, except after adrenalectomy or treatment with metyrapone; survival in these rats was 40–60%. The rate of urea nitrogen appearance by burned animals was 60% greater than that of unburned rats (Table I), which suggests the presence of increased protein and amino acid catabolism in vivo. At 48 h after thermal injury, plasma levels of glucagon were increased about fivefold but plasma catecholamines were not increased significantly. In burned rats, serum glucose was 24% higher, while the glycogen content of their epitrochlearis muscles was 52% lower. There was no difference in the cyclic AMP content of muscle between the two groups of rats.

In Table II are shown the rates of PS and PD in forelimb epitrochlearis muscles that were obtained 48 h after thermal injury; PD was increased 61% compared with that of muscles from unburned rats, while PS was unchanged. 10 mU/ml insulin raised PS and lowered PD in muscles from burned and unburned rats to a comparable degree. Despite this supraphysiologic concentration of insulin, PD in muscle from burned rats was still 72% higher than in muscles from unburned rats. To determine how this change in PD compared with the response to a direct thermal injury, a 3 s, scalding injury was administered to the upper limbs and chest (~20% of body surface area). After 48 h, PD in the underlying epitrochlearis muscles of these rats also was increased (209±8 nmol tyrosine/g per h), and 10 mU/ml insulin reduced it 20±4% to 181±7 nmol tyrosine/g per h.

To determine whether the proteolytic response was affected by the environmental temperature (42), six burned rats were housed in a ventilated chamber that was maintained at 32°C for the 48 h after the injury. PD in their epitrochlearis muscles was 224±11 nmol tyrosine/g per h and not different statistically from that of burned rats that were housed at room temperature.

Table I. Metabolic Responses to Thermal Injury

	Burned	Unburned
Initial weight (*g*)	197±6	194±5
Change in weight (g/48 h)	−33±3	−35±3
Serum urea nitrogen (*mg/ml*)	26.2±2.4	21.6±4.6
Urea appearance rate (mg N/100 g/48 h)	358±29‡	222±27
Serum creatinine (*mg/dl*)	0.36±0.03	0.28±0.03
Serum glucose (*mg/dl*)	140±7*	113±9
Plasma glucagon (*pg/ml*)	305±25‡	57±8
Plasma norepinephrine (*pg/ml*)	232±43	238±9
Plasma epinephrine (*pg/ml*)	274±84	407±88
Plasma dopamine (*pg/ml*)	47±6	55±7
Muscle protein (*mg/g*)	230±20	200±20
Muscle cyclic AMP (*pmol/g*)	505±41	451±24
Muscle glycogen (*μmol glucose/g*)	14.6±1.2‡	30.4±1.5

Values are expressed as mean±SEM from 6 to 10 rats. Serum chemical and plasma hormone levels were measured 48 h after beginning the experiment.
* $P < 0.05$.
‡ $P < 0.01$, compared with values in unburned rats.

Table II. Rates of PS and PD in Incubated Epitrochlearis Muscles from Burned and Unburned Rats

	PS (*nmol phe/g/h*)			PD (*nmol tyr/g/h*)		
	Basal	+Insulin	%Change	Basal	+Insulin	%Change
Burned	33±1	52±3	+51±8	225±5*	189±9*	−20±4
Unburned	34±2	54±3	+51±5	140±5	110±6	−22±5

Values are mean±SEM from muscles of 8–12 rats in each group that were incubated without (basal) and with 10 mU/ml insulin. PD was measured in the presence of 0.5 mM cycloheximide. Phe, phenylalanine; tyr, tyrosine.
* $P < 0.001$, compared with values from muscles of unburned rats.

In vivo studies. By treating rats in vivo, we investigated whether adrenal hormones, muscle prostaglandin synthesis, or lysosomal proteases were necessary for this proteolytic response (Table III). After eliminating adrenal steroids and catecholamines by adrenalectomizing (ADX) rats 24 h before the burn, we found that PD in muscle was still increased 58% by the burn. Dexamethasone (2 mg/kg per 12 h) increased PD in muscles of unburned-ADX rats, but the increase was only half as great as that induced by the burn (Table III). Lactate release from muscles of burned rats also was increased (9.55±0.82 μmol/g per h, burned, vs. 6.12±0.46 μmol/g per h, unburned; $P < 0.01$), and it remained high despite adrenalectomy (10.62±0.79 μmol/g per h, burn-ADX, vs. 6.81±0.64 μmol/g per h, unburned-ADX; $P < 0.01$). Dexamethasone, 2 mg/kg per 12 h, also increased lactate release from muscles of unburned-ADX rats (9.16±0.43 μmol/g per h), but it did not change muscle lactate release from burned rats (9.87±0.56 μmol/g per h, burn-ADX, steroid treated).

To examine the effect of selective glucocorticoid deficiency, glucocorticoid production was blocked by metyrapone injections (43). Metyrapone caused a small increase in the accelerated proteolysis induced by the burn ($P < 0.05$) and also tended to increase muscle proteolysis in unburned rats (Table III). However, PD in muscles from burned rats that were treated with metyrapone was still 58% greater than that in muscles of unburned rats. Finally, to examine the effects of circulating catecholamines, propranolol was injected at a dose that was sufficient to inhibit β-adrenergic effects (44). This regimen did not change PD in muscles from burned rats.

Table III. Results of In Vivo Experiments Designed to Assess Potential Mediators of Burned-induced Muscle Proteolysis

	PD (*nmol Tyr/g/h*)	
Treatment	Burned	Unburned
Untreated	231±7*	154±8
Adrenalectomy	215±6*	145±7
Adrenalectomy + dexamethasone (2 mg/kg/12 h, s.c.)	238±11*	186±5‡
Metyrapone (1 mg/kg/8 h, s.c.)	276±18*	174±9
Propranolol (2 mg/kg/6 h, s.c.)	242±7*	152±6
Indomethacin (3 mg/kg/8 h, i.p.)	249±8*	161±7
EP 475 (10 mg/kg/8 h, s.c.)	186±9§‖	152±10

Values are mean±SEM from incubated muscles of 5–10 rats in each treatment group. Adrenalectomy was performed 24 h before the experiment. Treatment schedules were begun 2 h before the burn. PD was measured in the presence of 0.5 mM cycloheximide. Tyr, tyrosine.

* $P < 0.01$, compared with muscles from unburned rats.

‡ $P < 0.01$, compared with muscles from unburned, adrenalectomized rats.

§ $P < 0.05$.

‖ $P < 0.01$, compared with untreated, burned rats.

Recent reports that increased muscle PGE_2 synthesis is associated with accelerated muscle proteolysis (36, 37) prompted us to examine the relationship between burn-induced muscle proteolysis and PGE_2 production. The rate of PGE_2 release by incubated muscles of burned rats was not different from that by muscles of unburned rats (22.1±2.8, burned, vs. 20.2±1.9 ng PGE_2/g per 2 h, unburned). Administration of indomethacin (3 mg/kg i.p.) to rats 2 h before and every 8 h after thermal injury reduced the rate of muscle PGE_2 release to 8.5±3.5 ng PGE_2/g per 2 h ($P < 0.01$) but did not alter the high rate of muscle PD (Table III). To exclude the possibility that incubation with cycloheximide might inhibit prostaglandin production, cycloheximide was omitted from the media. Again, the burn did not increase PGE_2 release from muscle (29.1±3.0, burned, vs. 26.6±3.0 ng/g per h unburned) and injection of indomethacin did not inhibit net proteolysis, even though PGE_2 release was reduced by 53%.

To investigate whether lysosomal proteases were necessary for this proteolytic response of muscle, we injected subcutaneously 10 mg/kg per 8 h of an oil emulsion of EP-475, which is an inhibitor of lysosomal thiol proteases (36–38, 45). This regimen reduced cathepsin B activity in epitrochlearis muscles from 99±4 to 8±4 nmol/g per min in burned rats and from 81±5 to 10±3 nmol/g per min in unburned rats. EP-475 injections reduced PD 20% ($P < 0.01$) in muscles from burned rats but did not change PD in muscle from unburned rats (Table III).

In vitro studies. Incubation with 1 μM epinephrine did not change PD in muscles of burned rats, but reduced it 15.8±3.6% ($P < 0.01$) in muscles of unburned rats (Table IV). Glucagon (10^7 pg/ml) increased PD in incubated muscles from fasted unburned rats by 78% but did not change the high rate of muscle PD present after a burn. A more physiologic concentration of glucagon (10^4 pg/ml) did not change PD or PS in muscles of normal, fasted rats. To examine whether glucocorticoids might increase the sensitivity of muscle PD to the acute effects of glucagon or epinephrine, normal rats were fasted and given 2 mg/kg per 12 h dexamethasone subcutaneously for 48 h. Muscles were then incubated with or without 0.1 μM epinephrine and/or 10^4 pg/ml glucagon (Table IV). Dexamethasone increased muscle PD 32% ($P < 0.01$), but there was no additional increase when epinephrine and/or glucagon were added to the media.

To examine the responsiveness of burn-induced muscle proteolysis to compounds that can inhibit protein degradation, muscles were incubated with insulin, α-ketoisocaproate (αKIC), indomethacin, and the thiol protease inhibitor, EP-475 (Table V). Insulin decreased the high rate of burn-induced PD by 20% and αKIC decreased it 28%. αKIC also decreased the rate of muscle lactate release from 8.64±0.64 to 3.84±0.32 μmol/g per h ($P < 0.01$). Indomethacin decreased PGE_2 release by incubated muscles of burned rats from 22.9±2.4 to 3.2±1.7 ng/g per 2 h ($P < 0.01$) but did not inhibit the high rate of PD. Similar conclusions were reached from results of incubations in which cycloheximide was omitted from the media.

Table IV. Effects of Epinephrine and Glucagon on PD in Incubated Muscles

Treatment	Experimental addition	PD (*nmol Tyr/g/h*) No addition	Addition	Percentage change
Fasting	Epinephrine (1 μM)	153±7	128±5	−15.8±3.6*
	Glucagon (10^4 pg/ml)	137±8	133±6	+1.6±8.1
	Glucagon (10^7 pg/ml)	144±8	268±18	+78.4±6.2*
Fasting + Dexamethasone	Epinephrine (0.1 μM)	203±5‡	201±7	−0.5±0.3
	Glucagon (10^4 pg/ml)	206±10‡	202±6	−1.1±2.7
	Epinephrine (0.1 μM) + Glucagon (10^4 pg/ml)	201±9‡	191±10	−5.2±3.6
Burn	Epinephrine (1 μM)	246±18‡	238±15	−0.9±3.7
	Glucagon (10^7 pg/ml)	225±5‡	225±11	+1.4±4.8

Results are expressed as mean±SEM. For each experiment, six rats were treated as indicated and the in vitro effects of the hormones on PD were measured in the presence of 0.5 mM cycloheximide. The effects of dexamethasone in fasted rats were studied by injecting groups of rats with 2 mg/kg per 12 h dexamethasone subcutaneously for 48 h before the incubation. Tyr, tyrosine.
* $P < 0.01$ for PD in muscles that were incubated without hormones compared with the rate in contralateral muscles that were incubated with hormones.
‡ $P < 0.01$, compared with values in muscles from untreated, fasted normal rats.

Incubation with EP-475 decreased PD by 14–18% and inhibited cathepsin B activity by >95%. The antiproteolytic effects of insulin and EP-475 were additive, reducing the high rate of burn-induced PD by 41% (Table V). Because insulin may decrease PD by inhibiting lysosomal proteolysis (46), we examined the dose-response relationship between EP-475 and muscle PD at 10 mU/ml insulin (Fig. 1). Insulin alone did not inhibit muscle cathepsin B activity, but insulin plus 25 μM EP-475 reduced it by 95%. With 50 μM EP-475, burn-induced muscle proteolysis was reduced to a rate comparable with that observed in muscles of normal, fasted rats that were incubated with insulin alone (Tables II and V).

Glucose metabolism. Because a high rate of lactate release from muscle can be associated with an accelerated rate of PD (17) (see above), we examined glucose metabolism in more detail. At a glucose concentration of 5 mM, the basal rate of 2-deoxy-U-^{14}C-glucose transport into muscle from burned rats was higher than into muscle of unburned rats (Table VI). However, when glucose was 10 or 20 mM, glucose transport into muscles from the two groups was not different either in incubations with or without 10 mU/ml insulin. Because of the potential difficulties associated with measuring 2-deoxy-glucose transport (47), we also calculated transport as the rate of ^3H_2O formed during phosphorylation of 2-^{3}H-glucose (24, 29). The results confirmed that the burn did not cause marked changes in glucose transport (Table VI).

We also measured the effect of thermal injury on different pathways of glucose utilization by incubating muscles with U-^{14}C-glucose and 5-^{3}H-glucose. In Table VII, the effects of 0, 100 μU/ml, and 10 mU/ml insulin on these pathways are presented. In the absence of insulin, total glucose uptake during the 2-h incubation was increased 82% in muscles from burned rats while net glycolysis and lactate release were increased 72 and 48%, respectively. Incubation with 100 μU/ml insulin stimulated GS in both groups of muscles to a comparable degree. Glucose uptake was higher in muscles of burned rats and there was a 71% increase in net glycolysis and an 87% increase in lactate release. GO was 52% greater in muscles of burned rats while Sc between fructose-6-phosphate and fructose-1,6-diphosphate in these muscles was 62% less.

Table V. Effects of Potential Inhibitors of Proteolysis on PD in Incubated Muscles from Burned and Unburned Rats

Addition	PD (*nmol Tyr/g/h*) Burned No addition	Addition	% Change	Unburned No addition	Addition	% Change
Insulin (10 mU/ml)	223±10	176±9	−20.1±3.9*	142±6	108±4	−20.7±3.5*
EP 475 (25 μM)	219±7	184±6	−14.2±5.1*	150±9	123±9	−18.7±3.3*
Insulin (10 mU/ml) +EP 475 (25 μM)	218±11	123±5	−41.1±3.3*	141±5	93±5	−32.4±4.8*
αKIC (0.5 mM)	244±7	174±7	−28.4±1.2*	145±4	118±5	−16.3±3.9*
Indomethacin (3 μM)	221±14	225±11	−0.4±5.5	154±8	139±5	−7.8±4.7

Values are mean±SEM of muscles from 6 to 12 rats in each group. Muscles were incubated with 0.5 mM cycloheximide, 10 mM glucose, and other additions as indicated. Tyr, tyrosine; EP 475, the lysosomal thiol protease inhibitor. * $P < 0.01$ by paired analysis.

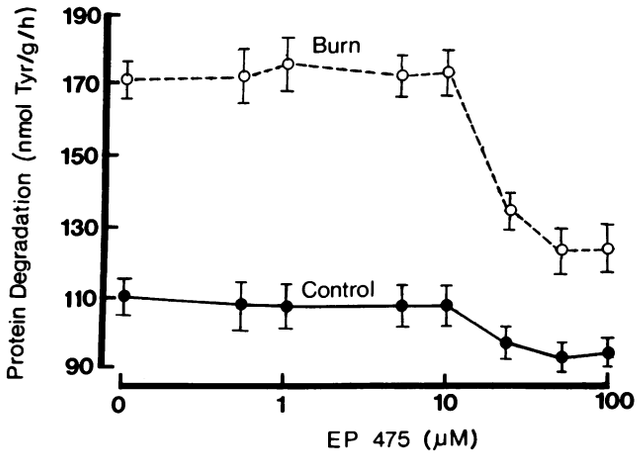

Figure 1. The dose-response relationship between PD in muscles that were incubated with 10 mU/ml insulin and different concentrations of EP-475 is shown. Results (mean±SEM) from muscles of groups of six burned (○) and six unburned (●) control rats are shown.

With 10 mU/ml of insulin in the media, GS was depressed 32% and net glycolysis and lactate release were increased 80–88%. GO was 119% greater than that of control muscles and again, Sc was depressed, proceeding at only one-fourth the rate measured in muscles from unburned rats.

In Fig. 2, the dose-response-relationships between insulin and rates of GS, net glycolysis, and GO in incubated muscles are shown. Thermal injury reduced the responsiveness (48) of GS to insulin without altering sensitivity to insulin. At all levels of insulin, net glycolysis and GO were greater in muscles from burned rats, but the insulin concentrations that yielded half-maximal responses were similar. As shown in Table VIII, abnormalities in insulin-stimulated glucose utilization that were induced by thermal injury persisted, even in adrenalectomized rats.

To examine whether the increase in muscle GO that was induced by the burn was associated with changes in the oxidation of exogenous fatty acids, muscles were incubated in a glucose-free media that contained 10 mU/ml insulin and 0.5 mM Na oleate plus [1-^{14}C]oleate (0.4 mCi/mmol). The rate of $^{14}CO_2$ that was released by muscles of burned rats (0.110±0.013 μmol/g per h) was not different statistically from that of muscles from unburned rats (0.106±0.010 μmol/g per h). The rates also were not different when 10 mM glucose was present in the media (0.174±0.011 μmol/g per h, burned; 0.189±0.015 μmol/g per h, unburned).

The net rate of muscle PD is shown in Table VII. This rate, the difference between PS and total PD, was significantly increased by thermal injury. Previously, we found that in a comparison of acutely uremic and control rats, net PD was increased in perfused muscle and was correlated with the proportion of glucose uptake which was released as lactate (17). We examined whether there was a similar relationship in the present study. As shown in Fig. 3, there was a significant, positive correlation ($r = 0.82$; $P < 0.001$), such that as the percentage of glucose uptake which was converted to lactate increased, the rate of net PD increased.

Discussion

This study demonstrates that a major thermal injury in rats causes accelerated protein and glucose catabolism in skeletal muscle that is distant from the burned area. After a scalding injury of the hindquarter, muscle PD was increased 60–70% in forelimb epitrochlearis muscles without a change in PS. The increase in PD was comparable with that induced by a burn of overlying tissues and was not affected by the environmental temperature (42) at which the rats were housed after

Table VI. Glucose Transport in Incubated Muscles from Burned and Unburned Rats

		− Insulin		+ Insulin	
	Glucose concentration	2-Deoxy-U-^{14}C-glucose	2-^{3}H-glucose	2-Deoxy-U-^{14}C-glucose	2-^{3}H-glucose
		nmol/g/min	*nmol/g/min*	*nmol/g/min*	*nmol/g/min*
Burned Rats	5 mM	220±36	188±39	267±21	220±14
	10 mM	327±33	300±49	494±11	407±21
	20 mM	667±18	633±25	801±21	705±24
Unburned Rats	5 mM	125±17	110±18	250±11	221±13
	10 mM	318±14	258±8	454±20	381±10
	20 mM	536±48	483±57	749±45	647±32

Values are mean±SEM. Five pairs of muscles from both burned and unburned rats were preincubated in glucose-free, Krebs-Henseleit buffer with or without 10 mU/ml insulin. Muscles were then transferred to flasks with fresh media that contained glucose, 2-deoxy-U-^{14}C-glucose, or 2-^{3}H-glucose with or without insulin and incubated for a subsequent 30 min. Glucose transport was determined as the amount of 2-deoxy-U-^{14}C-glucose in the intracellular space of muscle (26). Transport also was determined during incubation with 2-^{3}H-glucose by measuring the rate of $^{3}H_2O$ that was released during the interconversion of glucose-6-phosphate and fructose-6-phosphate (28).

Table VII. Glucose Metabolism and Net PD in Epitrochlearis Muscles from Burned and Unburned Rats Incubated with and without Insulin

		+ Insulin	
	Basal	100 μU/ml	10 mU/ml
Glucose uptake	C 2.606±0.243	10.545±1.141	25.423±1.222
(*μmol glucose/g/h*)	B 4.453±0.508*	15.491±1.422‡	30.822±1.197*
GS	C 0.363±0.052	4.550±0.872	12.755±0.836
(*μmol glucose/g/h*)	B 0.593±0.057*	4.679±0.399	8.713±0.637*
F-6-P ↔ F-1,6-diP	C 0.106±0.009	0.483±0.103	1.144±0.109
(*μmol glucose/g/h*)	B 0.095±0.001	0.184±0.057*	0.259±0.083*
Sc			
Net glycolysis	C 2.409±0.243	6.478±0.408	12.464±0.540
(*μmol glucose/g/h*)	B 4.153±0.471*	11.068±1.528*	22.486±1.236*
Lactate release	C 5.46±0.64	14.20±0.66	23.51±1.25
(*μmol/g/h*)	B 8.07±0.94*	26.34±2.09*	44.12±2.83*
GO	C 0.124±0.030	0.157±0.021	0.200±0.024
(*μmol glucose/g/h*)	B 0.161±0.014	0.239±0.027‡	0.438±0.014*
Net PD	C 127±8	113±9	82±5
(*nmol Tyr/g/h*)	B 198±14*	162±13*	158±8*

Values are expressed as mean±SEM in experiments using muscles from eight unburned (C) and eight burned (B) rats that were incubated with 10 mM glucose, U-^{14}C-glucose (5 μCi/mmol), and 5-^{3}H-glucose (0.1 mCi/mmol), as described in methods. Net glycolysis is the difference between the rates of $^{3}H_2O$ formation from 5-^{3}H-glucose and Sc.

* $P < 0.01$.

‡ $P < 0.05$, when compared with results obtained from control muscles.

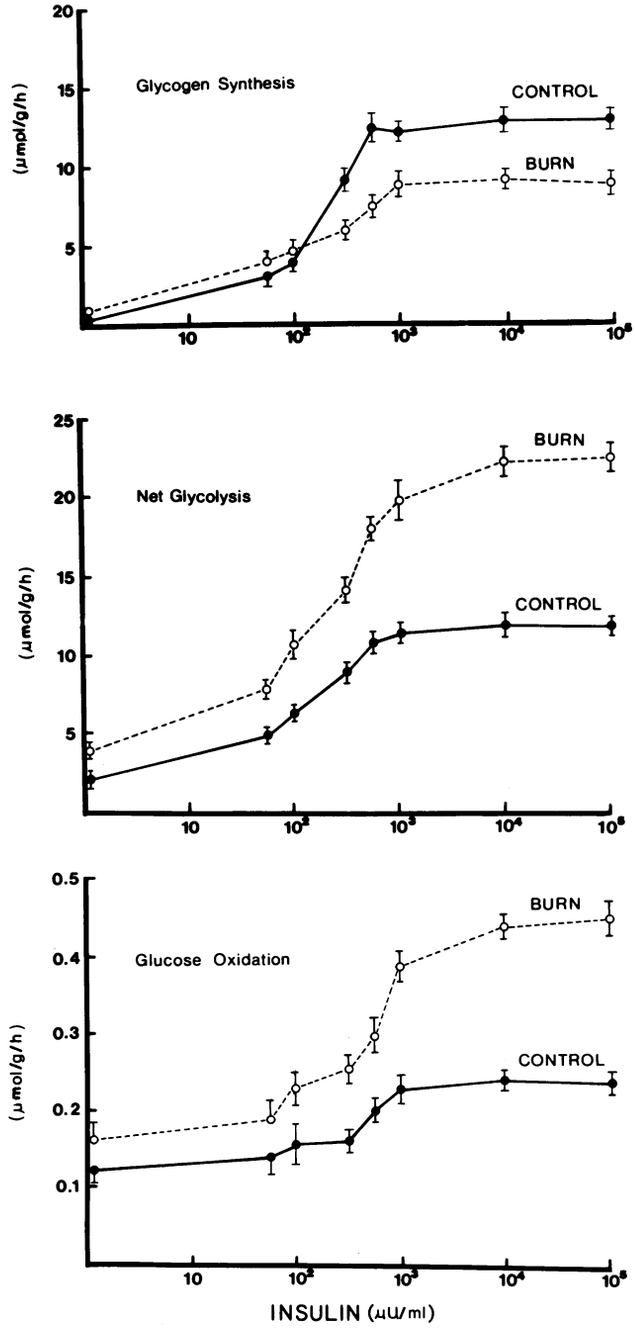

Figure 2. The dose-response relationships between GS, net glycolysis, and GO in incubated muscle and different concentrations of insulin are shown. Results are mean±SEM from muscles of groups of six burned (○) and six unburned (●) control rats studied at each insulin concentration.

their injury. In this model, accelerated proteolysis in mixed fiber muscles distant from the burn, as well as muscle underlying the burn, undoubtedly contributed to the 64% increase in the in vivo urea nitrogen appearance rate. This proteolytic response could not be attributed to adrenal catecholamines or corticosteroids, since muscle cyclic AMP levels were not increased by the thermal injury and PD in muscles of adrenalectomized or metyrapone-treated rats was 60–70% higher after a burn. Similarly, the increase in muscle PD did not require PGE_2 production in muscle, since a 53% inhibition of muscle PGE_2 production by injections of indomethacin and a >95% inhibition of PGE_2 release by incubation with indomethacin did not lower the high rate of muscle PD in burned rats. Interestingly, plasma glucagon was increased fivefold and incubation with a supraphysiologic concentration of glucagon (10^7 pg/ml) increased PD in muscles from fasted, normal rats to a level comparable with those that were induced by the burn (Tables I and IV). However, 10^4 pg/ml glucagon did not increase muscle PD of normal, fasted rats. To examine whether adrenal hormones might interact with glucagon to increase muscle PD, we pretreated normal, fasted rats with high doses of dexamethasone and then incubated their muscles with glucagon and/or epinephrine. We found no evidence that prolonged exposure to high doses of dexamethasone in vivo potentiated the proteolytic effects of glucagon and/or epinephrine (Table IV).

There are multiple pathways of protein breakdown in mammalian cells (45) that could contribute to the accelerated PD that occurs in response to a major burn. The results obtained with EP-475 (Table V, Fig. 1) establish the importance of lysosomal proteolysis. This compound enters cells and

Table VIII. Effects of Adrenalectomy on Insulin-stimulated Glucose Utilization in Incubated Epitrochlearis Muscles from Burned and Unburned Rats

	GS	Net glycolysis	GO
	μmol/g/h	*μmol/g/h*	*μmol/g/h*
Burn	9.16±0.32*	21.14±1.88*	0.57±0.03*
Burn (adrenalectomized)	6.15±0.63*	15.99±1.32*	0.62±0.05*
Control	12.27±0.95	13.84±1.24	0.28±0.02
Control (adrenalectomized)	8.41±0.47	9.62±0.52	0.30±0.02

Values are mean±SEM from muscles of five rats that were studied in each group 48 h after thermal or sham injury. Adrenalectomy or sham-operation was performed 24 h before injury. Muscles were incubated for 2 h with 10 mM glucose, 5-^{3}H-glucose (0.1 μCi/μmol), U-^{14}C-glucose (5 μCi/mmol), and 10 mU/ml insulin.
* $P < 0.02$, when compared with control muscles.

inhibits lysosomal thiol proteases (e.g., cathepsin B and perhaps H and L) without affecting the nonlysosomal ATP-dependent pathway or the calcium-activated protease (45). We found that EP-475 inhibited cathepsin B activity and suppressed burn-induced proteolysis. It has been suggested that insulin acts to suppress lysosomal proteolysis (46). The observation that the inhibitory effects of insulin and EP-475 were additive (Table V, Fig. 2) suggests that insulin might exert important antiproteolytic effects on nonlysosomal pathways. It seems unlikely that this additive effect was simply an insulin-stimulated increase in EP-475 uptake, since cathepsin B activity was almost completely inhibited by EP-475 when muscles were incubated without insulin (data not shown).

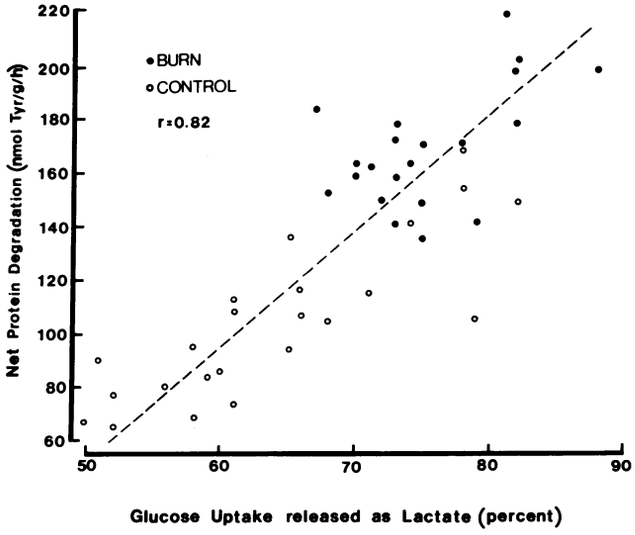

Figure 3. The relationship between net PD in muscles incubated with 50 μU/ml, 100 μU/ml, and 10 mU/ml insulin and the percentage of glucose uptake released as lactate is shown. Results from burned rats (•) and unburned rats (○) indicated that a significant correlation ($r = 0.82$) was present.

In addition to thermal injury, trauma also appears to cause a systemic, proteolytic response. Clowes et al. (13) reported that proteolysis was stimulated when normal rat soleus or human rectus abdominus muscles were incubated with plasma from traumatized patients. Purification studies indicated that a circulating glycoprotein could be the mediator of this response. Tischler and Fagan (49) have found that blunt trauma to the hindlimb of a rat causes nitrogen wasting, which was due in part to an increase in PD in uninjured soleus, extensor digitorum longus, and diaphragm muscles. As in our study, the increased PD in uninjured muscle was not corrected by insulin. In other ways, the response was different. Tischler and Fagan did not find that the rates of glycolysis and GO were increased in uninjured muscle.

In the present experiments, increased lactate release from muscle accompanied the accelerated proteolysis, which indicates that the catabolic response to thermal injury extended to glucose metabolism. We found that muscle glucose transport was unaffected by the burn (Table VI), but that total glucose utilization (glucose uptake) was increased both in the absence and presence of insulin (Table VII). It seems most likely that the increase in muscle lactate production was caused by accelerated glycolysis rather than by inhibition of pyruvate dehydrogenase activity, since GO in muscles from burned rats was increased at all levels of insulin studied. Insulin dose-response relationships (Fig. 2) indicated that thermal injury decreased the responsiveness (48) of GS to insulin. This could not be attributed to catecholamines (50, 51), since plasma catecholamine and muscle cyclic AMP levels were not elevated. We found no evidence that the sensitivity of muscle to insulin (48) was altered by the burn. Since glycolysis and GO were increased and Sc between fructose-6-phosphate and fructose-1,6-diphosphate was decreased, the yield of ATP per mole of glucose that was metabolized by muscle would have been substantially increased by the burn (25). Increased glucose catabolism did not appear to be caused by defective fatty acid metabolism, since oxidation of exogenous oleate was not impaired by the burn.

The correlation we found between net muscle PD and the percentage of insulin-stimulated glucose uptake directed into lactate production (Fig. 3) suggests that changes in glucose utilization and the rate of proteolysis in muscle are linked. The correlation does not prove that one abnormality necessarily caused the other defect, but there are several lines of evidence that suggest a link between increased lactate production from glucose and excessive PD in muscle. First, we found a similar correlation in a study of muscle metabolism in rats with another catabolic condition, acute renal failure (17). Second, when muscle PD was reduced by incubating with αKIC or increased by treating rats in vivo with high doses of dexamethasone, lactate release was decreased and increased, respectively.

Third, in humans, conditions such as uremia (52), sepsis (53, 54), trauma (55), and burns (3, 5, 6) are almost invariably associated with excessive lactate production and increased net urea production. A finding of potential therapeutic importance is the partial inhibition of both increased PD and lactate release in muscles of burned rats during incubation with αKIC. Unlike other branched-chain amino acids or their keto analogues, this compound can inhibit muscle protein degradation (56). Moreover, αKIC has been shown to improve nitrogen balance in starving, obese humans (57) and in patients who have undergone abdominal surgery (58). Thus, a study of the effects of αKIC on protein and carbohydrate metabolism in burned patients would be of interest. Moreover, an understanding of the relationship in Fig. 3 may lead to more rational therapy of conditions associated with excessive muscle proteolysis.

Acknowledgments

We wish to thank Dr. V. Barracos for measuring prostaglandin E_2, Dr. A. Friedlander at the National Institutes of Health Diabetes Core Lab, U. Virginia School of Medicine, Charlottesville, VA, for measuring cyclic AMP, and Drs. N. Sojka and E. Wright of the Div. of Comparative Medicine, U. Virginia School of Medicine, for their assistance in developing the protocol for thermal injury. G. Karapanos and L. Perry provided technical assistance, and P. Dolan provided expert editorial assistance.

This work was supported by grant AM 26903.

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