Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis

WEN LONG, EUGENE J. BARRETT, LIPING WEI, AND ZHENQI LIU
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908
Submitted 24 January 2002; accepted in final form 23 September 2002

Long, Wen, Eugene J. Barrett, Liping Wei, and Zhenqi Liu. Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis. Am J Physiol Endocrinol Metab 284: E102–E109, 2003. First published October 1, 2002; 10.1152/ajpendo.00028.2002.—After confirming that adre- nalectomy per se does not affect skeletal muscle protein synthesis rates, we examined whether endogenously pro-duced glucocorticoids modulate the effect of physiological insulin concentrations on protein synthesis in overnight-fasted rats 4 days after either a bilateral adrenalectomy (ADX); ADX with dexamethasone treatment (ADX + DEX), or a sham operation (Sham; n = 6 each). Rats received a 3-h euglycemic insulin clamp (3 mU·min⁻¹·kg⁻¹). Rectus mus- cle protein synthesis was measured at the end of the clamp, and the phosphorylation states of protein kinase B (Akt), eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), and ribosomal protein S6 kinase (p70S6K) were quantitated before and after the insulin clamp. The basal phosphoryla-tion states of Akt, 4E-BP1, and p70S6K were similar between ADX and Sham rats. Insulin significantly enhanced the phos-phorylation of Akt (P < 0.03), 4E-BP1 (P = 0.003), and p70S6K (P < 0.002) in ADX but not in Sham rats. Protein synthesis was significantly greater after insulin infusion in ADX than in Sham rats (P = 0.01). Glucocorticoid replace-ment blunted the effect of insulin on Akt, 4E-BP1, and p70S6K phosphorylation and protein synthesis. In conclusion, glucocorticoid deficiency enhances the insulin sensitivity of muscle protein synthesis, which is mediated by increased phosphorylation of translation initiation-regulatory proteins. Dexamethasone; translation initiation; protein kinase B; eukaryotic initiation factor 4E-binding protein-1; p70 S6 kinase (PI 3-kinase)-mammalian target of rapamycin (mTOR) pathway involved in the phosphorylation and activation of 4E-BP1 and p70S6K. It is downstream of PI 3-kinase and itself promotes the phosphorylation and activation of mTOR (4, 7, 48, 57). 4E-BP1, in its unphosphorylated form, functions as an mRNA translation initiation repressor by binding to eIF4E. Phosphorylation of 4E-BP1 frees eIF4E, which can then associate with eIF4G to initiate mRNA translation. Phosphorylation of p70S6K increases the phosphorylation of ribosomal protein S6 (44), which facilitates 5’-terminal oligopyrimidine mRNA translation and in-creases the synthesis of some ribosomal proteins, translation initiation factors, and elongation factors that play important roles in protein synthesis (28, 43).

Glucocorticoid excess induces negative nitrogen balance in experimental animals and humans by enhancing proteolysis, decreasing transport of amino acids into muscle, and inhibiting protein synthesis (10, 45, 54, 56). Glucocorticoid administration acutely inhibits muscle protein synthesis in rats as early as 4 h (46, 49, 55). The mechanism(s) underlying this effect on protein synthesis remains incompletely understood. Recent studies suggest that the major site for glucocorticoid’s negative action on protein synthesis is the inhibition of mRNA translation initiation (45, 49). In rats, high-dose dexamethasone treatment (100 µg/100 g body wt) acutely inhibits protein synthesis, decreases the phosphorylation of both 4E-BP1 and p70S6K, and lowers the amounts of eIF4E bound to eIF4G (49). Interestingly, more prolonged, but lower (though still pharmacological), doses of dexamethasone (2 mg every 6 h for 3 days) did not affect postabsorptive protein synthesis or the phosphorylation status of either 4E-BP1 or p70S6K in humans (31, 34). However, insulin and amino acids induced activation of 4E-BP1, and/or p70S6K was significantly impaired, both in rats (33) and in humans (31).

These observations have shed some light on the potential mechanisms for the interactions between the stimulatory effect of insulin and the inhibitory effect of glucocorticoids on protein synthesis. However, most studies have used pharmacological doses of insulin

Address for reprint requests and other correspondence: Zhenqi Liu, Dept. of Internal Medicine, Division of Endocrinology and Metabolism, PO Box 801410, Univ. of Virginia Health Sciences Center, Charlottesville, VA 22908–1410 (E-mail: z3e@virginia.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
and/or glucocorticoids, making the physiological significance of these findings less certain. Considering insulin, for example, many investigators have failed to demonstrate a stimulatory effect of physiological insulin concentrations on either whole body or skeletal muscle protein synthesis in adult rats or humans in vivo (1, 9, 16, 20, 36, 37, 39, 41, 59, 62). We have previously reported (22, 32) that physiological hyperinsulinemia stimulates the phosphorylation of p70S6K, but not 4E-BP1, in rat and human skeletal muscle. Whether physiological concentrations of glucocorticoids modulate the activation of the protein synthetic pathway by physiological concentrations of insulin has not been defined.

The major purpose of this study was to examine whether endogenous glucocorticoids modulate insulin-stimulated muscle protein synthesis at physiological stress concentrations in vivo and whether the signaling pathway that regulates protein synthesis via phosphorylation of Akt, 4E-BP1, and p70S6K is affected by adrenalectomy. We used an adrenalectomized rat model with or without glucocorticoid replacement to study the effect of insulin on protein synthesis pathway and the insulin signal transduction pathway. The results showed that insulin at physiological concentrations significantly stimulated skeletal muscle protein synthesis and increased the phosphorylation of Akt, 4E-BP1, and p70S6K in adrenalectomized rats compared with sham-operated animals. Dexamethasone, at a dose selected to replace endogenous glucocorticoids in adrenalectomized rats, blunted the muscle’s response to insulin. These findings suggest that endogenous glucocorticoids play a significant role in blunting insulin’s stimulatory effect on protein synthesis at physiologic concentration in vivo.

MATERIALS AND METHODS

Animal preparation and experimental protocols. Male Sprague-Dawley rats, weighing 225–250 g, were studied 4 days after either a bilateral adrenalectomy or a sham operation under general anesthesia using pentobarbital sodium (50 mg/kg ip; Abbott Laboratories, North Chicago, IL). Two separate studies were conducted.

In study 1, as a preliminary study, we examined whether adrenalectomy per se affects the rates of skeletal muscle protein synthesis in two groups of rats (n = 5 each). One group underwent adrenalectomy and the other group had sham operation. All surgeries were done through a midabdominal incision. In sham-operated rats, both adrenal glands were isolated and then left in place. After surgery, rats were maintained on a 12:12-h light-dark cycle with food and water provided ad libitum. Normal saline was provided instead of water to all adrenalectomized rats to prevent dehydration. Four days after surgery, rats were fasted overnight and then anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). The external jugular vein, internal carotid artery, and trachea were exposed and cannulated through a midline neck incision. The arterial catheter was connected through a three-way stopcock to a pressure probe. Heart rate and mean arterial pressure were monitored throughout the study (Transonic Systems, Ithaca, NY). Pentobarbital sodium was infused at a variable rate to maintain a steady level of anesthesia throughout the study. After a 45-min baseline period to assure hemodynamic stability and a stable level of anesthesia, all rats received a 3-h saline infusion, and a phenylalanine bolus (150 μmol/100 g body wt) containing L-[ring-2,6-3H]phenylalanine (375 μCi) was injected through the jugular vein 10 min before the completion of saline infusion. Gastrocnemius muscle was removed at the end of the infusion period and was freeze-clamped in liquid nitrogen. All muscle samples were stored at −70°C until analysis.

In study 2, three groups of rats were studied to examine the effect of adrenalectomy on insulin-stimulated translation initiation and protein synthesis. One group of rats (n = 6) had a bilateral adrenalectomy (ADX), another group of animals (n = 6) had a bilateral adrenalectomy and was placed on 5 μg/100 g body wt dexamethasone subcutaneously twice daily (ADX + DEX), and a third group of rats (n = 6) had sham operation (Sham). In ADX + DEX rats, the dose of dexamethasone was considered a physiological stress dose that approximates the glucocorticoid level found in stressed rats (8, 35). Four days after surgery, all rats were prepared as described for study 1. After a 45-min baseline period to assure hemodynamic stability and a stable level of anesthesia, all rats received a 3-h saline infusion, and a phenylalanine bolus (150 μmol/100 g body wt) containing L-[ring-2,6-3H]phenylalanine (375 μCi) was injected through the jugular vein 10 min before the completion of insulin infusion. Rectus muscle was again biopsied at the end of the infusion period and was freeze-clamped in liquid nitrogen. Both muscle biopsies were taken from lower abdominal rectus muscle that was not injured by wound surgery.

All muscle samples were stored at −70°C until analysis. Plasma insulin concentrations were measured by radioimmunoassay in plasma obtained at the beginning and the completion of the study. Plasma corticosterone concentrations were measured by radioimmunoassay in plasma obtained at the beginning of the study to confirm the success of adrenalectomy.

Measurement of skeletal muscle protein synthesis. Protein synthesis was estimated using a [3H]phenylalanine feeding technique as described previously (19). In brief, the phenylalanine concentration and the specific radioactivity in plasma and the muscle protein were measured by HPLC and β-counting. The protein synthesis rate is estimated from the radioactivity incorporated into the muscle protein over the 10-min period divided by the tracer specific activity and is presented as a fractional turnover rate (%/day). This method uses the tissue free amino acid pool as the precursor pool for the calculations. The phenylalanine bolus results in a rapid rise in specific radioactivity of phenylalanine in tissues, and two recent studies have demonstrated that the phenylalanine specific activity of this pool is similar to that of the true precursor pool in skeletal muscle in neonatal pigs (12) and dogs (5).

Western immunoblotting technique. Pieces (~30 mg) of frozen rectus muscle were weighed and powdered in frozen 25 mM Tris-HCl buffer (26 mM KF and 5 mM EDTA, pH 7.5). Tissue was then disrupted by sonication with the use of a microtip probe (0.5 s on and 0.5 s off for 45 s total) at a 3.0 power setting on the Fisher XL2020 sonicator (Fisher Scientific, Pittsburgh, PA). The homogenate was centrifuged at 2,000 rpm for 2 min, and the protein content of the superna-
tant was determined using the Bradford method (3). For Akt, one aliquot of the supernatant, containing ~60 μg of protein, was diluted with an equal volume of SDS sample buffer and electrophoresed on an 8% polyacrylamide gel. For 4E-BP1 and p70 S6K, one aliquot of the supernatant, containing ~50 μg of protein, was diluted with an equal volume of SDS sample buffer and electrophoresed on an 8% polyacrylamide gel. Proteins were then electrophoretically transferred to nitrocellulose membranes. After being blocked with 5% low-fat milk in Tris-buffered saline plus Tween 20, membranes were incubated with either rabbit polyclonal Akt antibody or phospho-Akt (Ser473) antibody (New England BioLabs, Beverly, MA) overnight at 4°C, or rabbit anti-4E-BP1 or p70S6K (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. This was followed by a donkey anti-rabbit IgG coupled to horseradish peroxidase, and the blot was developed using an enhanced chemiluminescence Western blotting kit (Amer sham Life Sciences, Piscataway, NJ).

Quantitation of Akt, 4E-BP1, and p70 S6K phosphorylation status. Autoradiographic films were scanned densitometrically (Molecular Dynamics, Piscataway, NJ) and quantitated using ImageQuant 3.3. Figure 1 illustrates the Akt, 4E-BP1, and p70 S6K phosphorylation status based on Western blot analysis. For Akt, both the total and phospho-Akt (Ser473) densities were quantitated, and the ratios of phosphospecific density to total density were calculated. To quantify the extent of phosphorylation of 4E-BP1, we measured the ratio of the intensity of the most slowly migrating species (γ) to that of the total intensity (α + β + γ). For p70 S6K, we determined the ratio of the more slowly migrating forms (β + γ) to the total (α + β + γ). Exploiting the different electrophoretic behaviors of proteins with various amounts of phosphorylation on SDS-PAGE allows the simultaneous quantification of multiple forms of both proteins, as well as internal normalization both for the recovery of target proteins from tissue and for loading of gels. The available data support a good correlation between activity and electrophoretic mobility. The α-band is the most phosphorylated form and moves the slowest. The β-band is phosphorylated more than the α-band and less than the γ-band, and shows up in between on SDS-PAGE. The Western blot shows the pattern from sham-operated (Sham; lanes 1 and 2), adrenalectomized (ADX; lanes 3 and 4), and ADX + treatment with dexamethasone (ADX + DEX; lanes 5 and 6) rats. Lanes 1, 3, and 5, basal period; lanes 2, 4, and 6, after insulin infusion.

Table 1. Characterization of Sham, ADX, and ADX + DEX animals in study 2

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>ADX</th>
<th>ADX + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>272 ± 6</td>
<td>248 ± 11</td>
<td>233 ± 8h</td>
</tr>
<tr>
<td>Food consumption, g/day</td>
<td>38 ± 4</td>
<td>24 ± 2.5</td>
<td>24.5 ± 5</td>
</tr>
<tr>
<td>Basal blood pressure, mmHg</td>
<td>117 ± 3</td>
<td>105 ± 4</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>Steady-state blood pressure, mmHg</td>
<td>106 ± 6</td>
<td>99 ± 3.4</td>
<td>103 ± 2.3</td>
</tr>
<tr>
<td>Basal insulin concentration, pmol/l</td>
<td>71 ± 5</td>
<td>38 ± 2</td>
<td>200 ± 17w</td>
</tr>
<tr>
<td>Steady-state insulin concentration, pmol/l</td>
<td>520 ± 26</td>
<td>548 ± 63</td>
<td>412 ± 31</td>
</tr>
<tr>
<td>Basal blood glucose, mM</td>
<td>5.3 ± 0.3</td>
<td>4.4 ± 0.2</td>
<td>5.1 ± 0.1</td>
</tr>
<tr>
<td>Steady-state blood glucose, mM</td>
<td>5.6 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Glucose infusion rate, mg·kg⁻¹·min⁻¹</td>
<td>9.9 ± 2.1</td>
<td>7.9 ± 0.9</td>
<td>6.0 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Sham, sham operated; ADX, adrenalectomized; DEX, dexamethasone. *P = 0.001 vs. Sham; †P = 0.01 vs. ADX; ‡P = 0.011 vs. Sham; §P = 0.022 vs. Sham; ¶P < 0.01 vs. Sham or ADX.

Statistical analysis. All data are presented as means ± SE. Statistical comparisons between the basal and insulin infusion periods were made using a two-tailed, paired t-test. Data comparisons among different groups were based on one-way ANOVA with post hoc testing or a two-tailed t-test. All statistical analyses were performed using SigmaStat 3.0 software.

RESULTS

Effect of adrenalectomy on basal protein synthesis. We first examined whether adrenalectomy affected the basal rate of skeletal muscle protein synthesis in animals in study 1. In this study, saline was infused, and the gastrocnemius muscle was used, as this is distant from the site of the initial surgical incision used for adrenalectomy and as repeated samples were not being taken for Western blotting. The fractional rates of protein synthesis in the gastrocnemius muscle were 4.3 ± 0.39 and 4.40 ± 0.66%/day in the ADX vs. Sham animals (P > 0.9). These findings suggest that adrenalectomy does not alter basal rates of skeletal muscle protein synthesis.

Characterization of study 2 experimental animals. After adrenalectomy, ADX rats consumed less food and weighed less than Sham rats (Table 1). The plasma corticosterone concentrations averaged 280 ± 55 ng/ml for Sham rats and were undetectable for both ADX and ADX + DEX rats, confirming the success of the adrenalectomy. The mean arterial blood pressures were not different among the groups either at baseline or during the insulin infusion period. Basal insulin concentrations were similar between Sham and ADX rats. Physiological stress dose glucocorticoid replacement increased the basal insulin concentrations nearly threefold. After euglycemic hyperinsulinemic clamp, insulin concentrations were compatible among all three groups of rats. The basal glucose concentrations were significantly lower in ADX rats than in Sham rats, and dexamethasone treatment eliminated this difference. During the insulin clamp, the steady-state
(120–180 min) glucose infusion rates required to maintain their blood glucose at their respective baseline were not different among all groups of rats.

Effect of adrenalectomy and insulin infusion on phosphorylation of Akt. The basal phosphorylation of Akt was comparable between Sham and ADX rats (0.212 ± 0.04 vs. 0.162 ± 0.03, respectively, P = 0.25). Glucocorticoid replacement slightly decreased basal phosphorylation state of Akt to 0.11 ± 0.02, though this was not statistically significantly different from Sham or ADX rats (P = 0.11 and 0.47 respectively). Insulin treatment significantly increased the phosphorylation of Akt in ADX rats by 112% (0.162 ± 0.03 vs. 0.345 ± 0.08, P < 0.03), but not in Sham rats. Dexamethasone treatment abolished this insulin-stimulated Akt phosphorylation in ADX + DEX rats (Fig. 2).

Effect of adrenalectomy and insulin infusion on phosphorylation of 4E-BP1. To quantitate the phosphorylation status of 4E-BP1, we calculated the ratio of the intensity of the most slowly migrating γ-band to that of the total integrated intensity (α + β + γ; Fig. 3). The γ-band is the highly phosphorylated form of 4E-BP1, and an increase in the quantity of this form would correspond to an increase in the phosphorylation of 4E-BP1 and a greater amount of eIF4E available to initiate translation. Basal phosphorylation status of 4E-BP1 was comparable among all three groups of rats, suggesting that adrenalectomy per se does not change the phosphorylation status of this protein. Physiological hyperinsulinemia did not stimulate 4E-BP1 phosphorylation in Sham rats (0.26 ± 0.02 vs. 0.29 ± 0.04, basal vs. insulin respectively, P = 0.24) but did significantly increase the phosphorylation status of 4E-BP1 in ADX rats (0.23 ± 0.02 vs. 0.39 ± 0.03, P = 0.003). Dexamethasone treatment blunted this increase in 4E-BP1 phosphorylation in ADX + DEX rats (0.26 ± 0.02 vs. 0.34 ± 0.06, P = 0.24).

Effect of adrenalectomy and insulin infusion on phosphorylation of p70\textsuperscript{S6K}. The extent of phosphorylation of p70\textsuperscript{S6K} was quantitated by measuring the ratio of the intensity of the more slowly migrating species (β + γ) to that of the total integrated intensity (α + β + γ; Fig. 4). The overall p70\textsuperscript{S6K} activity is dependent on the phosphorylation of at least seven Ser/Thr residues at three separate domains (44, 61). The more slowly migrating β- and γ-bands represent the more highly phosphorylated forms of p70\textsuperscript{S6K} and generally correspond to species with greater kinase activity. Similar to the effect of adrenalectomy on 4E-BP1 phosphorylation, adrenalectomy appears to have no effect on the baseline phosphorylation of p70\textsuperscript{S6K}, which was comparable among all three groups of rats. Insulin treatment did not stimulate p70\textsuperscript{S6K} phosphorylation in Sham rats (0.50 ± 0.06 vs. 0.46 ± 0.07, basal vs. insulin respectively, P = 0.42) but did significantly increase the phosphorylation status of p70\textsuperscript{S6K} in ADX rats (0.42 ± 0.05 vs. 0.63 ± 0.04, Fig. 4). Dexamethasone treatment again blunted this increase in p70\textsuperscript{S6K} phosphorylation in ADX + DEX rats (0.40 ± 0.09 vs. 0.49 ± 0.07, P = 0.24).

Effect of adrenalectomy on insulin-stimulated protein synthesis. Figure 5 illustrates the rates of protein synthesis observed in Sham, ADX, and ADX + DEX rats infused with insulin. After 3 h of euglycemic hyperinsulinemia, the skeletal muscle fractional protein synthesis rate in ADX rats was 30% higher than that in

Fig. 2. Effect of adrenalectomy and insulin infusion on Akt phosphorylation. Insulin infusion did not stimulate Akt phosphorylation in Sham control rats but significantly increased the phosphorylation of Akt in ADX rats. Dexamethasone treatment abolished this effect. *P < 0.03 vs. basal.

Fig. 3. Effect of adrenalectomy and insulin infusion on 4E-BP1 phosphorylation. Insulin significantly increased the phosphorylated portion of 4E-BP1 in ADX rats but not in Sham or ADX + DEX rats. *P = 0.003 vs. basal.

Fig. 4. Effect of adrenalectomy and insulin infusion on p70\textsuperscript{S6K} phosphorylation state. Insulin significantly stimulated p70\textsuperscript{S6K} phosphorylation in ADX rats but not in sham or ADX + DEX rats. *P < 0.002 vs. basal.
Glucocorticoid replacement with dexamethasone blunted fi
ticoid hormones induce synthesis of speci
sec steroids. Both glucocorticoids and catecholamines
alocorticoids, catecholamines, and small amounts of
signi
is not clear. In the present study, 3 h of physiological
glucocorticoids modulate the anabolic action of insulin

DISCUSSION

The adrenal glands produce glucocorticoids, miner-
allcorticoids, catecholamines, and small amounts of
sex steroids. Both glucocorticoids and catecholamines
antagonize insulin’s hypoglycemic effect. Mineralocor-
ticoid hormones induce synthesis of specific proteins
but have no known effect on bulk muscle protein me-
tabolism. Catecholamines acutely promote protein
anabolism by decreasing proteolysis (17, 29). It ap-
pears that the major adrenal hormones with negative
impact on protein synthesis are glucocorticoid hor-
mones. Glucocorticoids at pharmacological concen-
trations attenuate translation initiation and protein syn-
thesis (50–52) and blunt the anabolic action of insulin,
mixed amino acids, and branch-chain amino acids by
inhibiting the phosphorylation of 4E-BP1 and p70S6k
(31, 33). Whether physiological stress concentrations
of glucocorticoids modulate the anabolic action of insulin
is not clear. In the present study, 3 h of physiological
euglycemic hyperinsulinemia in adrenalectomized rats
significantly increased the skeletal muscle protein syn-
thesis and the phosphorylation of Akt, 4E-BP1, and
p70S6k, three key regulatory proteins in the insulin-
stimulated protein synthesis signal pathway, but had no
effect in sham-operated rats. Dexamethasone treat-
ment to replace physiological stress levels of glucocor-
ticoids blunted insulin’s action on all parameters ob-
served. These findings suggest that glucocorticoid
insufficiency produced by adrenalectomy enhances the
insulin sensitivity of muscle protein synthesis and that
endogenously present glucocorticoids can modulate in-
sulin’s anabolic action on muscle protein synthesis in
vivo.

At pharmacological doses, insulin has consistently
been shown to stimulate protein synthesis both in vivo
and in vitro (23–25, 38). A similar effect of physiolog-
ical hyperinsulinemia is consistently seen in immature
animals (11). Insulin activates the PI 3-kinase-mTOR
pathway and increases the phosphorylations of 4E-
BP1 and p70S6K (26, 33, 53). Within this pathway, Akt,
4E-BP1, and p70S6K are three proteins with pivotal
roles in insulin-stimulated protein synthesis. Akt is
activated in response to activation of PI 3-kinase by
phosphorylation at Thr308 and Ser473, and its activa-
tion is necessary for insulin-stimulated mTOR protein
kinase activity (48). Both 4E-BP1 and p70S6K are
downstream of Akt-mTOR in the insulin signal trans-
duction pathway, and phosphorylation of these two
proteins is critical in the initiation and maintenance of
protein synthesis. Phosphorylation of 4E-BP1 in-
creased the amount of eIF4E available for the forma-
tion of the translation initiation complex (30), a neces-
ary step for the translation of mRNAs with m7GTP at
the 5’ cap, and phosphorylation of p70S6K correlates
strongly with increases in ribosomal S6 kinase activity
(61), which appears to be necessary for maintaining the
apparatus required for ongoing protein synthesis. In
sham-operated rats, insulin did not stimulate protein
synthesis, nor did it activate the phosphorylation of
Akt, 4E-BP1, and p70S6K, confirming the absence of a
stimulatory effect of physiological hyperinsulinemia on
bulk protein synthesis in skeletal muscle in vivo. How-
ever, our results clearly demonstrated a significant
increase in protein synthesis after insulin administra-
tion in adrenalectomized rats. In accord with this in-
creased protein synthesis, insulin also increased the
phosphorylation status of Akt, 4E-BP1, and p70S6K in
adrenalectomized rats. These findings suggest that
adrenalectomized rats have heightened sensitivity to
the effect of physiological concentrations of insulin on
protein metabolism. Our observation of 4-day adrenal-
ectomy per se having no significant impact on the rates
of protein synthesis in the gastrocnemius muscle in
study 1 is entirely consistent with our findings of no
significant difference in the basal phosphorylation of
Akt, 4E-BP1, and p70S6K in the rectus among all treat-
ment groups in study 2. A previous report (35) has also
shown that the whole body protein synthesis rates are
especially the same in adrenalectomized rats and
adrenalectomized rats receiving stress dose dexameth-
asone (same dose as ours).

It is interesting that the glucose infusion rates were
not different among the three groups of rats studied.
Akt activation is thought to play a critical role in
insulin-stimulated GLUT4 translocation and glucose
uptake (57, 60). With significantly increased phosphor-
ylation of Akt in the ADX rats following insulin treat-
ment, one would expect that the glucose infusion rates
would be higher in this group of rats than in the Sham
rats. However, multiple confounding factors may have
contributed to the lack of higher glucose infusion rates
in the ADX rats in the present study. First, the basal
blood glucose concentrations were significantly lower
in ADX rats than in Sham rats, and the glucose was
infused to maintain the blood glucose concentrations at
their respective basal levels. Second, ADX rats have
lower body weight and less muscle mass, the site for
insulin-stimulated glucose disposal. Also, ADX rats,
being more stressed than Sham rats, may have higher levels of growth hormone, which is a counterregulatory hormone and antagonistic toward insulin's hypoglycemic effect. The elevated basal insulin concentrations and slightly lower, though not statistically significant, glucose infusion rate observed in ADX + DEX rats than in Sham and ADX rats are consistent with relative insulin resistance associated with physiological stress concentrations of glucocorticoids, a well-known clinical observation in patients with persistent and severe physical stress.

That dexamethasone treatment alone, administered at doses selected to replace glucocorticoids to physiological stress concentrations without mineralocorticoid or catecholamine replacement, blunted insulin-induced protein synthesis and phosphorylation of Akt, 4E-BP1, and p70S6K in adrenalectomized rats suggests that the increment in insulin sensitivity observed in adrenalectomized rats is secondary to endogenous glucocorticoid deficiency, not mineralocorticoid or catecholamine deficiency. Inasmuch as it has been previously observed that glucocorticoids given in excess blunt the action of amino acids (31, 33) and pharmacological doses of insulin (33) to stimulate protein synthesis, the present findings suggest that glucocorticoids modulate protein synthesis over the entire range of their availability. In addition, the present findings suggest that the presence of endogenous glucocorticoid hormones may be largely responsible for the lack of stimulatory effect of insulin on protein synthesis observed in most in vivo studies using physiological concentrations of insulin (1, 2, 6, 14, 18, 36, 37, 41).

We have previously observed (22, 32) that pharmacological doses of insulin enhanced the phosphorylation of both 4E-BP1 and p70S6K, whereas physiological hyperinsulinemia differentially phosphorylated p70S6K, but not 4E-BP1, in both human and rat skeletal muscle. In the present study, the physiological hyperinsulinemic clamp did not increase the phosphorylation of p70S6K in the sham-operated rats. The lack of effect of insulin on downstream signaling pathways in the sham-operated rats could be due, in part, to experimental conditions, including the involvement of increased concentrations of glucocorticoids. Abdominal surgery and prolonged anesthesia are associated with significant stress and increased endogenous production of glucocorticoid hormones sufficient to cause insulin resistance and may have diminished the response to insulin in sham-operated rats. Excessive glucocorticoids decrease total tyrosine phosphorylation of the insulin receptor and insulin receptor substrate 1 (IRS-1) as well as the protein content of IRS-1 (21), decrease the phosphorylation of both 4E-BP1 and p70S6K (50), and impair insulin-induced phosphorylation of 4E-BP1 and p70S6K in rats (33). Shah et al. (51) have demonstrated that p70S6K is more sensitive to inhibition by glucocorticoids under growth-promoting conditions than is 4E-BP1.

In the present study, we used the rectus muscle to assay the phosphorylation state of Akt, 4E-BP1, and p70S6K. Rectus muscle was selected as it allows repeated measurements from snip biopsies with minimal trauma to the other portions of the muscle. In the present study, there would be an additional concern, inasmuch as an abdominal incision was used for the adrenalectomy four days before the infusion study. However, we believe that this surgery per se had little impact on the results observed for the following reasons. 1) Each animal served as its own control to examine the phosphorylation status of signaling intermediates. 2) All animals, including the sham-operated animals, received the same abdominal surgery, and there were no differences in the basal phosphorylation status of these proteins among all groups. 3) Aseptic inflammation, as might be expected at the site of abdominal incision, does not appear to affect the phosphorylation of 4E-BP1 (58). Finally, 4) the muscle biopsies from the rectus were made at sites in the lower abdominal rectus muscle, making every effort to avoid previously incised areas of muscle.

In conclusion, the present results suggest an important action of physiological stress concentrations of glucocorticoids in the regulation of protein synthesis by physiological hyperinsulinemia. In particular, our results suggest that low doses of glucocorticoids are able to specifically block the activation by insulin of three proteins that regulate mRNA translation: Akt, 4E-BP1, and p70S6K. This action of endogenous glucocorticoids to counterpoise the effect of insulin on the phosphorylation of translation-regulatory proteins may modulate the effect of physiological doses of insulin to stimulate protein synthesis in vivo.

This work was supported by National Institutes of Health Grants RR-15540 (Z. Liu), DK-38578, and DK-54058 (E. J. Barrett).

REFERENCES


