Glucocorticoid-induced insulin resistance of protein synthesis is independent of the rapamycin-sensitive pathways in rat skeletal muscle

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Abstract

This study was designed to evaluate the role of p70 S6 kinase (p70S6K), p90 S6 kinase (p90RSK) and mitogen-activated protein (MAP) kinase pathways in the insulin resistance of muscle protein synthesis observed during glucocorticoid treatment. Dexamethasone treatment decreased the effect of insulin on protein synthesis activity, our results provide evidence that glucocorticoid-induced alterations in muscle protein synthesis regulation by insulin do not involve factors or kinases that are dependent on MAP kinase and/or p90RSK activity.

Introduction

Circulating levels of glucocorticoids are elevated in Cushing’s syndrome, infections, stress and various traumatic conditions and clinical treatments (Dujovne & Azarnoff 1975, Vaughan et al. 1982, Legaspi et al. 1985, Bondy 1985, Tessitore et al. 1993). The association of glucocorticoid excess and muscle atrophy is now well established, and the mechanisms underlying muscle wasting consistently involve inhibition of skeletal muscle protein synthesis and/or increased proteolysis (Tomas et al. 1979, Rannels & Jefferson 1980, McGrath & Goldspink 1982, Odedra et al. 1983, Kayali et al. 1987). However, glucocorticoid-induced muscle atrophy may result from both the steroid effect per se and/or modification of anabolic hormone action on protein metabolism (i.e. insulin). Indeed, steroids are also known to be potent diabetogenic agents resulting from both hepatic and peripheral resistance to the action of insulin (Amatruda et al. 1985). It is well known that the actions of insulin on muscle glucose uptake (Weinstein et al. 1995), glycogen synthesis (Leighton et al. 1987), protein synthesis (Southorn et al. 1990, Dardevet et al. 1998) and proteolysis (Louard et al. 1994, Dardevet et al. 1998) are altered after glucocorticoid treatment.

Cellular responses to insulin involve interaction of the hormone with its receptor and subsequent activation of intracellular signalling pathways. One of the earliest post-receptor events is phosphorylation of the insulin receptor substrate-1 (IRS-1) on tyrosine residues which in turn acts as a ‘docking protein’ to activate a number of different proteins, including phosphatidylinositol 3 kinase (PI3 kinase) and growth factor receptor-binding protein-2 (see Myers & White (1993) and Denton & Tavaré (1995) for reviews). The latter protein is thought to be linked to the activation of Ras, which subsequently activates the mitogen-activated protein (MAP) kinase pathways. PI3 kinase activation represents a general mechanism in muscle insulin signalling toward glucose metabolism (Okada et al. 1994, Cheatham et al. 1994, Yeh et al. 1995), protein synthesis (Dardevet et al. 1996, Thompson & Palmer 1998) or proteolysis (Dardevet et al. 1996, Thompson & Palmer 1998). The downstream events linked to PI3 kinase remain unclear but its activation is required for insulin stimulation of the p70 S6 kinase (p70S6K) in skeletal muscle (Dardevet et al. 1996) and various cells (Cheatham et al. 1994, Cross et al. 1994, Welsh et al. 1994). Interestingly, blunting the activation of p70S6K with rapamycin impaired the stimulatory effect of insulin on protein synthesis in rat skeletal muscle.
(Dardevet et al. 1996) as well as in C2C12 myoblasts (Palmer et al. 1997) suggesting involvement of this kinase in the regulation of muscle protein synthesis by insulin.

The mechanism by which steroids alter insulin action on skeletal muscle protein synthesis remains unclear. Indeed, the first cellular events involved in the action of insulin, i.e. a change in insulin receptor number (Block & Buse 1989, Giorgino et al. 1993, Saad et al. 1993), insulin receptor autophosphorylation (Block & Buse 1989, Giorgino et al. 1993, Saad et al. 1993) and tyrosine phosphorylation of IRS-1 (Giorgino et al. 1993, Saad et al. 1993) are not modified by glucocorticoid treatment. Up to now, the only significant effect of glucocorticoids was a decrease in the association/activation of PI3 kinase with IRS-1 in response to insulin (Giorgino et al. 1993, Saad et al. 1993). However, it is not known if such an alteration modifies activation of the p70S6K pathways or MAP kinase pathways by insulin. In an attempt to understand the origin of the insulin resistance of muscle protein synthesis after glucocorticoid treatment, we examined in the present study the action of dexamethasone (DEX) on the activity of selected intracellular protein kinases such as p70S6K (rapamycin-sensitive pathway), p90RSK and MAP kinases (rapamycin-insensitive pathway) in rat epitrochlearis muscle in vitro.

Materials and Methods

Animals

Young (4–5-week-old) male Sprague-Dawley rats were purchased from Ifa-Credo (L’Arbresle, France) and housed under controlled environmental conditions (temperature, 22 °C; 12 h dark period starting at 1800 h). They were given free access to commercial laboratory chow and water before the experiments were performed. Rats were randomly divided into a control and a DEX-treated group. DEX (a synthetic glucocorticoid analogue that does not bind to plasma binding proteins) was given daily (at 0900 h) via the drinking water. DEX concentration was adjusted every day on the basis of drinking water intake the day before. Animals received 2±1 mg/kg per day for 4 days. As DEX was reported to decrease food intake, the control group was pair-fed to the DEX-treated group. Food intake was maintained at similar levels in both the groups during the DEX treatment as shown in Table 1. Thus differences between pair-fed and treated groups do not originate from different food intakes. DEX induced a progressive weight loss of 20% over the treatment period and the pair-fed animals stopped growing (Table 1). Animals were anaesthetized with sodium pentobarbital (6±0 mg/100 g body weight) after an overnight fast. Epitrochlearis muscles were dissected intact for incubation (see below). Separate animal protocols were performed to examine the effects of the inhibitors tested and to assess kinase activities (16 animals per groups).

![Table 1 Body weight and food intake of pair-fed and DEX-treated rats](image)

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</table>

Effect of rapamycin and PD98059 on insulin-stimulated protein synthesis

The effect of the inhibitors was assessed by preincubating epitrochlearis muscle for 30 min in Krebs/Henseleit buffer (NaCl 120 mM, KCl 4±8 mM, NaHCO3 25 mM, CaCl2 2±5 mM, KH2PO4 1±2 mM and MgSO4 1±2 mM; pH 7±4) supplemented with 5 mM Hepes, 5 mM glucose, 0±17 mM leucine, 0±20 mM valine, 0±10 mM isoleucine and 0±1% BSA (99% fatty acid free) and saturated with 95% O2/5% CO2 gas mixture in the presence or absence of 200 nM rapamycin or 20 µM PD98059. Muscles were then transferred to fresh medium of the same composition supplemented with 0±5 mM 1±[14C]phenylalanine (0±15 µCi/ml) with or without 30 mM insulin and incubated for an additional 90 min period. At the end of the incubation, muscles were blotted and homogenized in 10% trichloroacetic acid (TCA). Samples were centrifuged at 10 000 g for 10 min at 4 °C and TCA-insoluble material was washed three times with 10% TCA. The resultant pellet was solubilized in 1 M NaOH at 37 °C for determination of protein and radioactivity incorporated into muscle protein. Tissue protein mass was determined using the bicinechonic acid procedure (BCA; Pierce Chemical Co., Rockford, IL, USA), and protein-bound radioactivity was measured using scintillation counting. Protein synthesis was calculated by dividing the protein-bound radioactivity by the specific radioactivity of the phenylalanine in the incubation medium. It was expressed as nmol phenylalanine incorporated/µg protein per 90 min.

Assessment of p70S6K and p90RSK activities

Epitrochlearis muscles were preincubated for 30 min in Krebs/Henseleit buffer supplemented with 5 mM Hepes, 5 mM glucose, 0±17 mM leucine, 0±20 mM valine, 0±10 mM isoleucine and 0±1% BSA (99% fatty acid free) and saturated with 95% O2/5% CO2 gas mixture. Insulin (30 nM) was then added to the medium and muscles were incubated for 20 min in the presence of the hormone. Muscles were homogenized in ice-cold extraction buffer (50 mM Tris/acetate, 50 mM NaF, 2±5 mM EDTA, 1 mM EGTA, 5 mM sodium pyrophosphate, 5 mM ATP and 0±1% BSA (99% fatty acid free). Homogenates were centrifuged at 10 000 g for 20 min at 4 °C and the supernatant was used for analysis of p70S6K and p90RSK activities.

![Table 1 Body weight and food intake of pair-fed and DEX-treated rats](image)
**Results**

**Effect of DEX on insulin-stimulated muscle protein synthesis**

Glucocorticoid treatment induced skeletal muscle protein wasting in both epitrochlearis muscle (22.9 ± 1.2 vs 16.7 ± 0.6 mg in pair-fed and treated rats respectively) and gastrocnemius muscle (774 ± 3 vs 542 ± 2 mg in pair-fed and treated rats respectively). In pair-fed animals, muscle protein synthesis was significantly stimulated (P<0.05) by insulin (0.248 ± 0.0057 vs 0.489 ± 0.026 nmol phenylalanine/mg protein per 90 min without and with insulin respectively). Food restriction did not significantly modify the effect of insulin on epitrochlearis protein synthesis when compared with a control *ad libitum* group (0.295 ± 0.023 vs 0.485 ± 0.048 nmol phenylalanine/mg protein per 90 min without and with insulin respectively).

Insulin was still able to stimulate protein synthesis (0.327 ± 0.013 nmol phenylalanine/mg protein per 90 min; P<0.05 vs untreated rats) (Fig. 1). This result shows that the MAP kinase pathway is not involved in the acute regulation of muscle protein synthesis by insulin and thus could not be responsible for the defective hormone action under steroid treatment. To check the efficiency of the inhibitor, we assessed, in a side experiment, insulin-stimulated glucose transport. PD98059, at the concentration used above, completely inhibited the effect of insulin on muscle deoxyglucose (1.859 ± 0.107 vs 2.108 ± 0.273 nmol deoxyglucose/mg muscle per 15 min without and with insulin in the presence of PD98059; 1.661 ± 0.023 vs 0.485 ± 0.048 nmol phenylalanine/mg protein per 90 min without and with insulin respectively).

**Effect of PD98059 on insulin-stimulated protein synthesis**

Addition of PD98059 (an inhibitor of MAP kinase pathway) to the incubation medium did not modify the rate of basal muscle protein synthesis (0.248 ± 0.006 vs 0.243 ± 0.010 0.05 nmol phenylalanine/mg protein per 90 min without or with the inhibitor respectively) nor the stimulatory effect of insulin in both pair-fed and Dextreated rats (Fig. 1). Indeed, the incremental increase in protein synthesis above basal due to the effect of insulin was significantly decreased by 35.2% (P<0.05) when animals were treated with DEX (0.241 ± 0.010 vs 0.156 ± 0.005 nmol phenylalanine/mg protein per 90 min for pair-fed and DEX–treated rats respectively).

**Statistical analysis**

Data are expressed as the means ± s.e.m. Statistical evaluation of the data was performed using Student’s *t*-test. Differences among means were considered significant when *P*<0.05.
Effect of DEX on muscle insulin-stimulated p90RSK and p70S6K activities

Insulin stimulated the activity of p90RSK in epitrochlearis muscle from pair-fed rats (3·01 ± 0·170 vs 2·07 ± 0·256 pmol ATP/50 µg per 20 min with and without insulin respectively; P<0·05) (Fig. 2). Treatment with DEX modified neither the basal activity of p90RSK nor its stimulation by insulin (2·04 ± 0·165 vs 3·23 ± 0·181 pmol ATP/50 µg per 20 min with and without insulin respectively; P<0·05) (Fig. 2). Quantification of total p90RSK by Western blot revealed that the amount of kinase was not altered by the DEX treatment in epitrochlearis muscle (Fig. 2).

Stimulation of p70S6K by insulin is characterized by an increase in its apparent molecular mass secondary to multiple phosphorylation sites of the kinase. Therefore we used this property (assessed by Western blot) as an indicator of hormone action on the kinase. DEX treatment did not modify the amount of p70S6K protein recovered from epitrochlearis muscle (Fig. 3). The presence of insulin in the incubation medium greatly increased the phosphorylation of p70S6K in pair-fed animals whereas no modification of the electrophoretic mobility was recorded in DEX-treated epitrochlearis muscle (Fig. 3). This observation suggests that the stimulation of p70S6K activity by insulin was impaired when rats were treated with steroids. The measurement of p70S6K activity confirmed this observation as insulin greatly increased p70S6K activity in pair-fed animals (3·44 ± 0·55 vs 0·71 ± 0·18 pmol ATP/100 µg per h with and without insulin respectively; P<0·05) whereas it had no significant stimulatory effect in DEX-treated rats (0·78 ± 0·12 vs 0·56 ± 0·12 pmol ATP/100 µg per h with and without insulin respectively) (Fig. 3). It is important to note that DEX did not modify the basal activity of p70S6K in epitrochlearis muscle. This result suggests that the rapamycin pathway to which p70S6K belongs may be in part responsible for the defect in insulin action on muscle protein synthesis in DEX-treated rats. In contrast with protein synthesis, food restriction greatly modifies both basal and insulin-stimulated p70S6K in epitrochlearis muscle when compared with a control ad libitum group (1·296 ± 0·054 vs 7·010 ± 0·820 pmol ATP/100 µg per h without and with insulin respectively). Thus, in order to assess the effect of DEX alone on the intracellular pathways involved in the stimulation of muscle protein synthesis by insulin, it is essential to compare the DEX group with a pair-fed group.

Effect of DEX on muscle protein synthesis.

Epitrochlearis muscles from pair-fed (PF) and DEX-treated (DEX) rats were preincubated for 1 h in Krebs/Henseleit buffer supplemented with 5 mM Hepes, 5 mM glucose, 0·17 mM leucine, 0·2 mM valine, 0·1 mM isoleucine and 0·1% BSA and saturated with 95% O2/5% CO2 gas mixture in the presence or absence of 20 µM PD98059. Muscles were then transferred to fresh medium of the same composition supplemented with 0·5 mM N-acetylphenylalanine (0·15 µCi/ml) with or without 30 nM insulin and incubated for an additional 90 min period. Muscles were blotted and homogenized in 10% TCA. The resultant pellet was solubilized in 1 M NaOH at 37 °C for determination of muscle protein content and radioactivity incorporated into muscle proteins. Protein synthesis was expressed as nmol phenylalanine incorporated/mg protein per 90 min. Data are means ± s.e. for six to eight individual muscles. *Significantly different from basal value (P<0·05); †significantly different from the pair-fed value (P<0·05).

Figure 1 Effect of PD98059 on muscle protein synthesis. Epitrochlearis muscles from pair-fed (PF) and DEX-treated (DEX) rats were preincubated for 1 h in Krebs/Henseleit buffer supplemented with 5 mM Hepes, 5 mM glucose, 0·17 mM leucine, 0·2 mM valine, 0·1 mM isoleucine and 0·1% BSA and saturated with 95% O2/5% CO2 gas mixture in the presence or absence of 20 µM PD98059. Muscles were then transferred to fresh medium of the same composition supplemented with 0·5 mM N-acetylphenylalanine (0·15 µCi/ml) with or without 30 nM insulin and incubated for an additional 90 min period. Muscles were blotted and homogenized in 10% TCA. The resultant pellet was solubilized in 1 M NaOH at 37 °C for determination of muscle protein content and radioactivity incorporated into muscle proteins. Protein synthesis was expressed as nmol phenylalanine incorporated/mg protein per 90 min. Data are means ± s.e. for six to eight individual muscles. *Significantly different from basal value (P<0·05); †significantly different from the pair-fed value (P<0·05).

Figure 2 Activity of p90RSK in rat skeletal muscle. Epitrochlearis muscles were preincubated for 30 min in Krebs/Henseleit buffer. Muscles were then incubated for 20 min in the presence or not of 30 nM insulin. Muscles were homogenized in ice-cold extraction buffer and centrifuged at 10 000 g for 10 min at 4 °C. The activity of p90RSK was assessed by an immune complex kinase assay. After a 20 min incubation at 30 °C, the reaction mixture was spotted on Whatman P-81 phosphocellulose filter paper squares and counted. Aliquots of tissue proteins were analysed by SDS/PAGE (7·5% gel) under reducing conditions, transferred to a nylon membrane and exposed to a polyclonal antibody that specifically recognizes p90RSK. Data are means ± s.e. for eight to ten individual muscles. *Significantly different from basal value (P<0·05).
Effect of rapamycin on insulin-stimulated protein synthesis

The effect of rapamycin was assessed in an additional experiment in which the decrease in basal muscle protein synthesis and the defective action of insulin on muscle protein synthesis (-39.9%) was similar to the previous study (Fig. 4). Addition of rapamycin to the incubation medium did not modify basal protein synthesis in epitrochlearis muscle from pair-fed animals (0·241 ± 0·013 vs 0·233 ± 0·019 nmol phenylalanine/mg protein per 90 min without and with inhibitor respectively). Insulin increased protein synthesis but in the presence of rapamycin its effect was significantly reduced, by 0·071 ± 0·004 nmol phenylalanine/mg protein per 90 min (0·459 ± 0·020 vs 0·388 ± 0·015 nmol phenylalanine/mg protein per 90 min respectively) (Fig. 4). Thus insulin partially stimulated muscle protein synthesis through a rapamycin-dependent pathway.

As observed with pair-fed animals, the addition of rapamycin did not modify basal protein but partially inhibited the insulin-stimulated protein synthesis in DEX-treated rats. This inhibition was similar to that recorded in pair-fed animals: 0·069 ± 0·002 nmol phenylalanine/mg protein per 90 min (from 0·293 ± 0·019 to 0·224 ± 0·015 nmol phenylalanine/mg protein per 90 min with and without rapamycin respectively) (Fig. 4). This result shows that the insulin effect that is dependent on the rapamycin-sensitive pathway is not significantly altered in response to DEX and thus not responsible for the resistance observed.

Discussion

Glucocorticoids have direct effects on muscle protein turnover by either inhibiting protein synthesis (Tomas et al. 1979, Rannels & Jefferson 1980, McGrath & Goldspink 1982, Odedra et al. 1983, Kayali et al. 1987) or increasing proteolysis (Tomas et al. 1979, McGrath & Goldspink 1982, Kayali et al. 1987). However, in vivo, they also act through their interaction with insulin. Accordingly, our study in rat epitrochlearis muscle showed that DEX significantly decreased basal muscle protein synthesis but also strongly depressed its stimulation by insulin. The effect of insulin on muscle protein metabolism in vivo is still an open question. Most of the experiments...
demonstrated that infusion of exogenous insulin does not increase muscle protein synthesis except in young fasted growing rats (Garlick & Grant 1988, Mosoni et al. 1993). Thus it has been postulated that physiological concentrations of insulin already maximally stimulate protein synthesis in vivo. This was confirmed by the experiment of Sinaud et al. (1999) which demonstrated, in standard postprandial rats, that only a specific decrease in insulinemia produced by diazoxide was able to show the role of insulin in the stimulation of muscle protein synthesis in vivo. The in vitro stimulation of protein synthesis by insulin in epitrochlearis muscle occurred in the range zero to physiological insulin concentration and thus confirms the previous hypothesis (Dardevet et al. 1994, Vary et al. 1998). In our experiment we used a dose of insulin that stimulated protein synthesis maximally (as in vivo) and we postulate that we were reproducing insulin stimulation that occurs in vivo. As physiological insulin levels already maximally stimulate muscle protein synthesis in vivo, the difference in insulin-stimulated protein synthesis that we observed in vitro should also be found in vivo. The results of both Odedra et al. (1983) and Southorn et al. (1990) agree with this. They demonstrated that the responsiveness of muscle protein synthesis to insulin was altered when rats were treated with steroids and that insulin infusion in vivo did not counteract the decrease in muscle protein synthesis.

Cellular responses to insulin involve interaction of the hormone with its receptor and subsequent activation of intracellular signalling pathways. It is now established that phosphorylation/dephosphorylation reactions play a major role in the control of protein synthesis by insulin. The involvement of PI3 kinase as a mediator of insulin action on muscle protein synthesis has been demonstrated in studies using a specific inhibitor of the kinase (Dardevet et al. 1996, Thompson & Palmer 1998). However, PI3 kinase represents a general mechanism in insulin signalling; for example, several studies have shown that this kinase is also important in the regulation of glucose uptake (Okada et al. 1994, Cheatham et al. 1994, Yeh et al. 1995, Dardevet et al. 1996). The downstream events linked to PI3 kinase remain poorly defined but in several cell lines (Cheatham et al. 1994, Cross et al. 1994, Welsh et al. 1994, Chung et al. 1994) as well as in rat skeletal muscle (Dardevet et al. 1996), PI3 kinase activation was found to be required for insulin stimulation of p70S6K. Interestingly, using rapamycin, we previously showed that p70S6K (a rapamycin-sensitive kinase) may be partially involved in the signalling pathway linking the insulin receptor to the protein synthesis machinery in rat epitrochlearis muscle, as in the presence of the inhibitor, insulin stimulation of protein synthesis was decreased by 25% (Dardevet et al. 1996). In the present study, DEX treatment totally abolished the stimulation of p70S6K by insulin in rat epitrochlearis muscle without decreasing the enzyme content. Therefore such a modification could explain the insulin resistance of the muscle protein synthesis in DEX-treated rats. In order to verify whether such an alteration was indeed responsible for the glucocorticoid-induced insulin resistance of protein synthesis, we compared the effect of rapamycin on insulin-stimulated protein synthesis between pair-fed and DEX-treated rats. Surprisingly, the addition of rapamycin decreased the effect of insulin to the same extent in both groups of rats suggesting that the rapamycin-sensitive pathway was not involved in the insulin resistance observed in spite of total blockage of the stimulation of p70S6K by insulin. However, DEX-induced modification of the insulin-stimulated p70S6K activity may explain the alteration in other metabolic effects of insulin in skeletal muscle. Indeed DEX treatment decreased the action of insulin on glucose transport (Weinstein et al. 1995) or glycogen synthesis (Leighton et al. 1987) in which the involvement of a rapamycin-sensitive mechanism (including elements of the p70S6K signalling pathway) has been demonstrated (Azpiazu et al. 1996). Furthermore, insulin activates nuclear p70S6K (Kim & Kahn 1997) which has been shown to regulate the GLUT-1 and hexokinase II gene expression (Osawa et al. 1996, Taha et al. 1995). Thus, by inhibiting the p70S6K activation by insulin, DEX may indirectly modify glucose metabolism but not protein synthesis.

Although rapamycin causes a deactivation of p70S6K, the direct target of this inhibitor is in fact the protein known as mTOR (mammalian target of rapamycin). The role of mTOR as the rapamycin target responsible for inhibition of p70S6K was demonstrated by Brown et al. (1995) who showed that mutant TORs, which lack the ability to bind rapamycin, prevent the inhibition of p70S6K by the drug. However, recent studies (Hara et al. 1997, Von Manteuffel et al. 1997) demonstrated that rapamycin-resistant mutants of p70S6K are not able to prevent the rapamycin-dependent dephosphorylation of eukariotic initiation factor 4E binding protein 1 (eIF4E-BP1), a target of insulin in the regulation of protein synthesis. Thus, even if mTOR is an upstream regulator of the p70S6K, other signalling pathways arise directly from mTOR and act independently of the p70S6K. Our experiment is consistent with this observation, as we showed in DEX-treated rats that rapamycin was still able to inhibit the stimulation of muscle protein synthesis by insulin in the absence of stimulation of p70S6K. As the lack of stimulation of p70S6K by insulin in DEX-treated animals did not modify the rapamycin-associated decrease in protein synthesis, the involvement of p70S6K in the regulation of muscle protein synthesis by insulin was questionable. Furthermore, as food restriction altered the stimulation of p70S6K by insulin without having any effect on muscle protein synthesis, this observation supports our hypothesis that this kinase may not be directly involved in the regulation of muscle protein synthesis by insulin.

Insulin activates multiple cellular protein kinases (Myers & White 1993). These include MAP kinases which are...
part of a signal-transduction pathway that is distinct from the rapamycin-sensitive pathway. Indeed, it has been shown that rapamycin does not block the activation of MAP kinase by insulin or the downstream kinase p90RSK (Chung et al. 1992, Price et al. 1992, Fingar et al. 1993). Furthermore, because PI3 kinase activation was required for insulin stimulation of muscle protein synthesis (Dardevet et al. 1996, Palmer et al. 1997), and because wortmannin (inhibitor of PI3 kinase) has been shown to block the activation of MAP kinase by insulin in L6 myocytes (Cross et al. 1994) and CHO cells (Welsh et al. 1994), the role of this pathway in the DEX-induced insulin resistance of muscle protein synthesis was assessed in our model. Addition of the MAP kinase (MEK) inhibitor did not modify the stimulatory effect of insulin on muscle protein synthesis, suggesting that the MAP kinase pathway and its downstream elements were not involved in the regulation of the translation machinery. Our observations are in agreement with the study of Azpiazu et al. (1996) who concluded that the MAP kinase pathway was not necessary for insulin action on muscle protein synthesis in rat diaphragm. In consequence, the resistance of muscle protein synthesis to insulin in DEX-treated rats could not originate from a defect in this pathway. Furthermore, we showed that the stimulation by insulin of a downstream kinase of the MAP kinase pathway (p90RSK) was not altered by DEX treatment. However, the role of p90RSK in the regulation of muscle protein synthesis has been proposed because it activates the glycogen synthase kinase 3 which is known to activate eIF2B (eukaryotic initiation factor 2B) in vitro (Welsh & Proud 1993). The latter is a target of insulin in its regulation of the formation of the 43S preinitiation complex (Kimball et al. 1994). However, DEX-induced insulin resistance of muscle protein synthesis was also independent of p90RSK as treated animals did not show any alteration in the stimulation of this kinase by insulin. Further studies are nevertheless necessary to confirm whether or not p90RSK represents an insulin-stimulated kinase leading to stimulation of muscle protein synthesis.

Insulin modulates protein synthesis by enhancing initiation of translation of mRNA (Kimball et al. 1994). The rate-limiting step for translation is the initiation phase which involves recognition of capped mRNA and binding to the 40S ribosome (Aftmann & Trachsel 1993, Rhoads 1993, Sonenberg 1994). These processes require the participation of the initiation factor eIF4F, a complex composed of eIF4E (the cap binding protein), eIF4G and eIF4A (RNA helicase). eIF4E is the least abundant of the initiation factors and thus plays a critical regulatory role in protein synthesis. Modulation of its concentration by gene transfection leads to parallel modification of protein synthesis (Rhoads 1993, Sonenberg 1994). eIF4E activity is modulated by a protein known as 4E-BP-1 (also termed PHAS-I) which specifically binds to eIF4E and decreases translation (Lin et al. 1994, Pause et al. 1994).

Phosphorylation of PHAS-I in response to insulin resulted from a dissociation of the PHAS-I–eIF4E complex and thus increased free eIF4E for the formation of the complex eIF4F (Lin et al. 1994, 1995, Graves et al. 1995). To our knowledge, the effect of steroids on the insulin regulation of the complex PHAS-I–eIF4E has not been studied but we hypothesize that such a mechanism may occur in the generation of the insulin resistance of muscle protein synthesis in our model. Indeed, such a mechanism has been recently demonstrated in diabetes type I, another well-characterized insulin-resistant state. Interestingly, even if PHAS-I represents a substrate of MAP kinase in 3T3-L1 adipocytes (Lin et al. 1995) or rat diaphragm (Azpiazu et al. 1996) and was only partially blocked by rapamycin (Azpiazu et al. 1996). Further work will be required to demonstrate the contribution of such a mechanism in the generation of the DEX-induced insulin resistance of protein synthesis in rat epitrochlearis muscle.

In conclusion, our experiments show that glucocorticoid-induced insulin resistance of muscle protein synthesis is independent of the rapamycin-sensitive pathway despite a total inhibition of p70RSK activation by insulin. In addition, our results on the p90RSK and MAP kinases show that they also were not responsible for the insulin resistance observed. Investigations are needed to determine which intracellular kinases or factors are involved. From previous (Dardevet et al. 1996) and the present results, it was found that that these kinases are dependent on PI3 kinase but independent of rapamycin and MAP kinase. Protein kinase B (PKB) may be a good candidate because its activation by insulin was blocked by LY294002 (inhibitor of the PI3 kinase) and unaffected by rapamycin and PD98059 (inhibitor of MAP kinase pathways; see Cohen et al. (1997) for a review). Furthermore, this kinase has been linked to the glycogen synthase kinase 3 in skeletal muscle (Cohen et al. 1997). Whether or not PKB is responsible for the insulin resistance of protein synthesis in DEX-treated rats remains to be determined.

References


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