Glucagon Represses Signaling through the Mammalian Target of Rapamycin in Rat Liver by Activating AMP-activated Protein Kinase*

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The opposing actions of glucagon and insulin on glucose metabolism within the liver are essential mechanisms for maintaining plasma glucose concentrations within narrow limits. Less well studied are the counter-regulatory actions of glucagon on protein metabolism. In the present study, the effect of glucagon on amino acid-induced signaling through the mammalian target of rapamycin (mTOR), an important controller of the mRNA binding step in translation initiation, was examined using the perfused rat liver as an experimental model. The results show that amino acids enhance signaling through mTOR resulting in phosphorylation of eukaryotic initiation factor 4E-binding protein (4E-BP)1, the 70-kDa ribosomal protein (rp)S6 kinase, S6K1, and rpS6. In contrast, glucagon repressed both basal and amino acid-induced signaling through mTOR, as assessed by changes in the phosphorylation of 4E-BP1 and S6K1. The repression was associated with the activation of protein kinase A and enhanced phosphorylation of LKB1 and the AMP-activated protein kinase (AMPK). Surprisingly, the phosphorylation of two S6K1 substrates, rpS6 and eukaryotic initiation factor 4E, was not repressed but instead was increased by glucagon treatment, regardless of the amino acid concentration. The latter finding could be explained by the glucagon-induced phosphorylation of the ERK1 and the 90-kDa rpS6 kinase p90rsk. Thus, glucagon represses phosphorylation of 4E-BP1 and S6K1 through the activation of a protein kinase A-LKB-AMPK-mTOR signaling pathway, while simultaneously enhancing phosphorylation of other downstream effectors of mTOR through the activation of the extracellular signal-regulated protein kinase 1-p90rsk signaling pathway. Amino acids also enhance AMPK phosphorylation, although to a lesser extent than glucagon and amino acids combined.

Received for publication, September 17, 2004, and in revised form, October 18, 2004
Published, JBC Papers in Press, October 19, 2004, DOI 10.1074/jbc.M410755200

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This paper is available on line at http://www.jbc.org

* This study was supported by National Institutes of Health Grant DK-13499. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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§ The abbreviations used are: rp, ribosomal protein; mTOR, mammalian target of rapamycin; eIF, eukaryotic initiation factor; BP, binding protein; AMPK, AMP-activated protein kinase; PKA, protein kinase A; ERK, extracellular signal-regulated kinase; MOPS, 4-morpholinepropanesulfonic acid.

This paper is available on line at http://www.jbc.org 54103
tion of cAMP in response to glucagon would initiate a cascade of events starting with the activation of PKA, LKB1, and AMPK and ending in decreased mTOR signaling.

An alternative mechanism through which glucagon may repress signaling through mTOR involves the activation of the mitogen-activated protein kinase-signaling pathway. In support of this possibility, glucagon activates the extracellular signal-regulated kinase (ERK)-signaling pathway in cells in culture (22, 23). Moreover, previous reports have shown that activation of either the ERK or p38 mitogen-activated protein kinase-signaling pathway results in phosphorylation of tuberin and that inhibition of ERK signaling prevents the epidermal growth factor-mediated activation of S6K1 (24–26). Mitogen-activated protein kinases also phosphorylate the 90-kDa rpS6 protein kinase, p90<sup>rbk</sup> (reviewed in Ref. 27). However, although p90<sup>rbk</sup> phosphorolizes rpS6 in <i>in vitro</i> reactions, its role as an rpS6 kinase in <i>in vivo</i> has been questioned (reviewed in Ref. 28). Thus, glucagon may repress mTOR signaling through either the AMPK or mitogen-activated protein kinase signaling pathways or both.

In the present study, the effects of glucagon on basal and amino acid-induced signaling through mTOR were examined. The results showed that glucagon repressed amino acid-induced phosphorylation of two downstream targets of mTOR signaling, 4E-BP1 and S6K1. Moreover, glucagon stimulated phosphorylation of AMPK, LKB1, ERK1/2, and p90<sup>rbk</sup> independent of amino acid concentration. Surprisingly, glucagon also stimulated phosphorylation of the S6K1/S6K2 substrates rpS6 and eIF4B, suggesting that p90<sup>rbk</sup> may function to phosphorylate the two proteins under these conditions. Overall, the results demonstrated that glucagon repressed amino acid-induced signaling through mTOR but that activation of the ERK1/2 signaling pathway supersedes the inhibition of S6K1 and was associated with an increase in phosphorylation of proteins traditionally considered to be targets of S6K1.

**Experimental Procedures**

**Materials**—Sodium pentobarbital was purchased from Abbot. Bovine albumin (essentially fatty acid free), glucagon, and protease inhibitor mixture were purchased from Sigma. ECL and ECL Western blotting detection reagents were purchased from GE Healthcare, and polyvinylidene difluoride membrane (BioTrace, 0.45 μm) was purchased from Pall Life Sciences. BioMag goat anti-rabbit polyclonal IgG beads were purchased from Qiagen. Rabbit polyclonal antibodies raised against LKB1, ERK1/2, and p90<sup>rbk</sup> were purchased from Cell Signaling Technologies. The rabbit polyclonal antibodies that specifically recognize phosphorylation of Ser-428 on LKB1, Thr-389 on S6K1, Ser-1108 on eIF4G, Thr-172 on AMPK, Ser-422 on eIF4B, Thr-202/Tyr-204 on ERK1/2, Ser-380 on p90<sup>rbk</sup>, and Ser-235/Ser-236 and Ser-240/Ser-244 on rpS6 were also purchased from Cell Signaling Technologies. Rabbit polyclonal antibodies against S6K1 and 4E-BP1 and the goat anti-rabbit IgG horseradish peroxidase-conjugated antibody were purchased from AbD Serotec Laboratories. Preparation of the eIF4B antibody has been described previously (29).

**In Situ Liver Perfusion**—Male Sprague-Dawley rats weighing 100–130 g at the time of experimentation were maintained on a 12-h light/12-h dark cycle with food (Harlan Teklad), and water was provided ad libitum (the experimental protocol used for the studies described herein was reviewed and approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine). Prior to experimentation, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital at 6 mg/100 g of body weight. Livers were perfused in situ with a non-recirculating medium at a flow rate of 7 ml/min, as described previously (30, 31). The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 5 mM glucose, 3% w/v bovine albumin, 33% washed bovine erythrocytes, and amino acids at 1 or 4× the concentrations found in the arterial plasma of postabsorptive rats (32). The perfusion medium was maintained at 37 °C and gassed with a mixture of 95% O<sub>2</sub>, 5% CO<sub>2</sub>. When present, glucagon (10 nm in 0.155 M NaCl (pH 3.0)) was infused directly into the inflow line at a rate of 0.2 ml/min for a final calculated concentration of 0.3 nm. Total perfusion time was 10 min. Immediately following perfusion, livers were excised and frozen between aluminum blocks that were precooled in liquid nitrogen, and the frozen liver was stored at −80 °C for future analysis.

**Measurement of PKA Activity**—The phophotransferase activity of PKA was measured using a PKA assay kit (Upstate Cell Signaling Solutions). For this purpose, 0.2 g of frozen liver tissue was homogenized in 7 volumes of 1× Assay Dilution Buffer consisting of 20 mM MOPS (pH 7.2), 25 mM β-glycerophosphate, 5 mM EGTA, 1 mM sodium vanadate, 0.1 mM dithiothreitol, 1 μM microcystin, and 10 μM/l Sigma protease inhibitor mixture. The resulting homogenate was centrifuged at 1000 × g for 3 min at 4 °C, and the protein concentration in the supernatant was determined using a kit from Bio-Rad. Supernatant containing 50 μg of protein was diluted to 5 μl with 1× Assay Dilution Buffer followed by the addition of 25 μl of assay mix (16 mM MOPS (pH 7.2), 20 mM β-glycerophosphate, 4 mM EGTA, 0.8 mM sodium vanadate, 80 mM dithiothreitol, 0.8 μM microcystin, 5 μM/l Sigma protease inhibitor mixture, 100 μM Kemptide, 7.5 mM magnesium chloride, 100 μM [γ<sup>32</sup>P]ATP (1 μCi/μmol), 0.4 μM PKC inhibitor peptide, and 4 μM compound R24571 (a calmodulin-dependent protein kinase inhibitor)). A duplicate sample of diluted supernatant was assayed using the same assay mix containing 1 μM PKA inhibitor peptide. Both assay mixtures were incubated at 30 °C for 5 min, at which time 15 μl of each of the assay mixtures was spotted onto PS1 phosphocellulose filters. The filters were washed subsequently with 0.75% phosphoric acid and acetone, as recommended in the manufacturer's instructions to the PKA assay kit, and the amount of radioactivity bound to the filters was measured by liquid scintillation spectrometry. PKA activity was calculated as the difference between the sample assayed in the absence of PKA inhibitor peptide and the sample assayed with the inhibitor.

**Western Blot Analysis**—A portion (0.3–0.4 g) of frozen liver was homogenized in 7 volumes of buffer consisting of 20 mM HEPES, 2 mM EGTA, 50 mM NaF, 100 mM KCl, 0.2 mM EDTA, 50 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM benzamidine, 0.5 mM sodium vanadate, and 10 μM/l Sigma protease inhibitor mixture using a Polytron homogenizer. The homogenate was centrifuged immediately at 1000 × g for 3 min at 4 °C after which 200 μl of supernatant was added to an equal volume of 2× SDS sample buffer and quenched with 5% glycerol. The samples were then stored at −80 °C until analyzed. The electrophoretic mobility of the proteins analyzed in this study was determined by SDS-polyacrylamide gel electrophoresis using the following acrylamide concentrations: 7.5% for S6K1 and eIF4G, 10% for LKB1, AMPK, and eIF4B, 12.5% for rpS6, ERK1/2, and p90<sup>rbk</sup>, and 15% for 4E-BP1. After electrophoresis, the proteins were transferred electrophoretically to a polyvinyldiene difluoride membrane that was then blocked for 1 h in a solution of 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween (pH 7.6). The membrane was then incubated overnight at 4 °C with primary antibody followed by the next day by a 1-h incubation in secondary antibody. The blots were developed using an ECL or ECL+ Western blotting kit as recommended by the manufacturer. The chemiluminescent signal was quantified using a stainless steel X-ray film and high-speed Gnome Bioimaging system (Syngene). Membranes probed with a phosho-specific primary antibody were then reprobed with an antibody that recognizes the protein independent of phosphorylation status after removing the phospho-specific antibody, according to the procedure recommended by GE Healthcare (TechTips no. 120).

**Measurement of Protein Phosphorylation State**—Phosphorylation of S6K1, eIF4G, eIF4B, AMPK, 4E-BP1, ERK1/2, p90<sup>rbk</sup>, and rpS6 was measured in the supernatants centrifuged at 1000 × g by Western blot analysis as described above. Phosphorylation of LKB1 was measured in 1000 × g supernatants that had been cleared by preincubation with BioMag beads.

**Results**

One of the best characterized actions of glucagon is elevation of intracellular cAMP and subsequent activation of PKA (33). Therefore, PKA activity was used as an index of the effectiveness of the dose of the hormone and time of exposure utilized in the present study. As shown in Fig. 1, PKA activity was unaffected by changes in perfusate amino acid concentration. However, PKA activity was markedly greater in extracts of livers perfused in the presence of glucagon than livers perfused in the absence of the hormone.

To assess amino acid- and glucagon-induced changes in signaling through mTOR, the phosphorylation of two mTOR substrates, 4E-BP1 and S6K1, was assessed. As shown in Fig. 2,
phosphorylation of 4E-BP1 was enhanced in livers perfused with medium containing 4× compared with 1× amino acids. In contrast, glucagon repressed both basal and amino acid-induced phosphorylation of 4E-BP1. Similarly, phosphorylation of S6K1 was enhanced in livers perfused with 4× compared with 1× amino acids when assessed by either hyperphosphorylation of the protein (Fig. 3A) or phosphorylation of a specific residue, Thr-389 (Fig. 3B). Glucagon had no effect on basal S6K1 phosphorylation but repressed amino acid-induced phosphorylation of the protein. Thus, the data support the idea that glucagon attenuates amino acid-induced signaling through mTOR.

In isolated hepatocytes, the amino acid-induced phosphorylation of S6K1 is attenuated by activation of AMPK using 5-aminimidazole-4-carboxamide 1-β-D-ribofuranoside (14, 15). Moreover, activation of PKA in 3T3-L1 adipocytes by either forskolin or isoproterenol enhances phosphorylation of AMPK on Thr-172 (34). Therefore, in the present study, the effect of glucagon treatment on AMPK phosphorylation was assessed. As shown in Fig. 4, perfusion of livers in the presence of 4× amino acids

FIG. 1. Effect of amino acids and glucagon on PKA activity in perfused rat liver. Rat livers were perfused in situ with medium containing amino acids at concentrations found in the plasma of postabsorptive rats (1× amino acids (AA) (32)) or 4× those concentrations), as described under “Experimental Procedures.” When present, glucagon was infused directly into the inflow at a final calculated concentration of 0.3 nM. PKA activity was assessed as described under “Experimental Procedures,” and results are expressed as disintegrations/min (DPM) incorporated into the peptide substrate/50 μg of protein in the assay and represent the mean ± S.E. (n = 8–9 livers/condition). Light gray bars, livers perfused without glucagon; dark gray bars, livers perfused with glucagon. *, p < 0.001 versus livers perfused with 1× amino acids without glucagon; †, p < 0.001 versus livers perfused with 4× amino acids without glucagon.

FIG. 2. Effect of amino acids and glucagon on 4E-BP1 phosphorylation in perfused rat liver. Rat livers were perfused as described in the legend to Fig. 1, and 4E-BP1 phosphorylation was assessed by changes in migration during SDS-polyacrylamide electrophoresis, as described under “Experimental Procedures.” The results are expressed as the proportion of the protein present in the hyperphosphorylated γ-form and represent the mean ± S.E. (n = 8–9 livers/condition). Light gray bars, livers perfused without glucagon; dark gray bars, livers perfused with glucagon. Results of a typical blot are presented in the inset. The α-, β-, and γ-forms of 4E-BP1 are denoted to the right. Lane 1, livers perfused with 1× amino acids (AA) without glucagon; lane 2, livers perfused with 1× amino acids and glucagon; lane 3, livers perfused with 4× amino acids without glucagon; and lane 4, livers perfused with 4× amino acids and glucagon. *, p < 0.02 versus livers perfused with 1× amino acids without glucagon; **, p < 0.001 versus livers perfused with 1× amino acids without glucagon; †, p < 0.0005 versus livers perfused with 4× amino acids without glucagon.

FIG. 3. Effect of amino acids and glucagon on phosphorylation of S6K1 in perfused rat liver. Rat livers were perfused as described in the legend to Fig. 1. A, hyperphosphorylation of S6K1 was assessed as the decreased migration of the protein during SDS-polyacrylamide gel electrophoresis. A typical blot is shown in which 8–9 livers per condition were analyzed. –, without glucagon; +, with glucagon. B, phosphorylation of S6K1 on Thr-389 was assessed by Western blot analysis as described under “Experimental Procedures.” The results represent the mean ± S.E. (n = 8–9 livers per condition). Light gray bars, livers perfused without glucagon; dark gray bars, livers perfused with glucagon. Results of a typical blot are presented in the inset. Lane 1, livers perfused with 1× amino acids (AA) without glucagon; lane 2, livers perfused with 1× amino acids and glucagon; lane 3, livers perfused with 4× amino acids without glucagon; and lane 4, livers perfused with 4× amino acids and glucagon. **, p < 0.0001 versus livers perfused with 1× amino acids without glucagon; †, p < 0.002 versus livers perfused with 4× amino acids without glucagon, and p < 0.02 versus livers perfused with 1× amino acids without glucagon.
resulted in a 2-fold increase in the relative phosphorylation of AMPK on Thr-172 compared with livers perfused in the presence of 1× amino acid. Similarly, glucagon treatment increased AMPK(Thr-172) phosphorylation 2-fold in the presence of 1× amino acids and further enhanced the phosphorylation of the protein in livers exposed to 4× amino acids.

To examine the possible involvement of the AMPK protein kinase LKB1 in the activation of AMPK by glucagon, the effects of amino acids and glucagon on LKB1 phosphorylation on Ser-428, a residue phosphorylated by PKA in vitro (20), was assessed. As shown in Fig. 5, increasing amino acids from 1 to 4× had no effect on LKB1 phosphorylation, whereas glucagon significantly enhanced phosphorylation of Ser-428 on LKB1 independent of perfusate amino acid concentration.

To further examine changes in signaling through mTOR, the phosphorylation state of two proteins downstream of S6K1, rpS6 and eIF4B, was assessed. Increasing perfusate amino acid concentrations from 1 to 4× had no significant effect on the phosphorylation of eIF4B (Fig. 6) or of rpS6 on Ser-235/Ser-236 (Fig. 7A), but phosphorylation of rpS6 on Ser-240/Ser-244 was enhanced (Fig. 7B). Glucagon had no significant effect on rpS6/Ser-240/Ser-244 phosphorylation regardless of perfusate amino acid concentration. Surprisingly, glucagon enhanced the phosphorylation of both eIF4B and rpS6(Ser-235/Ser-236) in the presence of either 1 or 4× amino acids, suggesting that the hormone activates a kinase that can phosphorylate these two S6K1 substrates.

Because p90<sub>ras</sub> is also a rpS6 kinase, the effect of glucagon on p90<sub>ras</sub> phosphorylation was examined. As shown in Fig. 8A, the phosphorylation of p90<sub>ras</sub> was unaffected by changes in perfusate amino acid concentration. However, treatment with glucagon resulted in a decrease in migration of the kinase during SDS-polyacrylamide gel electrophoresis, suggesting that glucagon enhanced the phosphorylation of p90<sub>ras</sub>. Similarly, amino acids had no effect on the phosphorylation of the p90<sub>ras</sub> upstream kinase ERK1 or ERK2 (Fig. 8B), whereas exposure to glucagon caused a decrease in ERK1 but not ERK2 migration during SDS-polyacrylamide gel electrophoresis, suggesting that glucagon promotes activation of ERK1 in perfused rat liver.

**DISCUSSION**

In the present study, the hypothesis that glucagon represses amino acid-induced signaling through mTOR was tested using the perfused rat liver as an experimental model system. Previous studies in vivo (35–37) and in isolated hepatocytes (14–16) have demonstrated that amino acids and, in particular, the branched-chain amino acid leucine promote signaling through mTOR, as assessed by phosphorylation of the mTOR substrates 4E-BP1 and S6K1. In the present study, we confirmed the results of the earlier studies and showed that increasing perfusate amino acid concentrations from those found in plasma of postabsorptive rats to four times those levels results in enhanced phosphorylation of both 4E-BP1 and S6K1, indicating that amino acids promote signaling through mTOR in perfused rat liver. We extended the earlier studies to show that co-administration of glucagon with amino acids prevents the amino acid-induced phosphorylation of 4E-BP1 and S6K1, supporting the hypothesis that glucagon represses amino acid-induced signaling through mTOR. The hypothesis is also supported by the results from a recent study (38) that was published while the present study was being prepared for submission. In that study, it was found that glucagon attenuates...
amino acid- and insulin-induced phosphorylation of 4E-BP1 and S6K1 in isolated rat hepatocytes and that the effect of glucagon on mTOR signaling appears to be independent of the phosphorylation of mTOR on Ser-2448, because all three treatments, amino acids, insulin, and glucagon, promote phosphorylation of that site. However, in that study, the signal transduction pathway involved in the effects of glucagon on mTOR was not examined further.

To our knowledge, there are no reports describing the pathway through which glucagon may modulate signaling through mTOR. However, although it was not recognized in this context at the time, when viewed in hindsight, a number of studies have provided evidence suggesting a link between glucagon acting through PKA to repress mTOR signaling. Perhaps the first clue supporting this idea was the discovery that mutations in the gene encoding the protein kinase LKB1 are causative in Peutz-Jeghers syndrome (reviewed in Refs. 39 and 40). Peutz-Jeghers syndrome is characterized by the development of multiple hamartomatous polyps in the gastrointestinal tract as well as the development of tumors in other tissues. The development of hamartoma in multiple tissues is also characteristic of tuberous sclerosis, a disease linked to mutations in the genes encoding the proteins tuberin and hamartin (reviewed in Refs. 41 and 42), suggesting that LKB1 and tuberin/hamartin may function through similar mechanisms to regulate cell growth. Tuberin is a GTPase-activating protein for the small G protein referred to as Ras homolog enriched in brain (Rheb) (reviewed in Refs. 43 and 44). Recent studies have reported that Rheb is a positive regulator of mTOR signaling and that its overexpression enhances phosphorylation of 4E-BP1 and S6K1 in an mTOR-dependent manner (45, 46). Thus, by promoting GTP hydrolysis on Rheb, tuberin inhibits signaling through mTOR.

The missing link between LKB1 and tuberin was provided by two groundbreaking studies. The first study (19) demonstrates that LKB1 phosphorylates and thereby activates AMPK, and the second study (47) shows that AMPK phosphorylates and thereby activates tuberin. The final piece of the puzzle appears to be the finding that PKA directly phosphorylates LKB1 on Ser-428 (Ser-431 in the human sequence) and that this residue has an essential role in the control of cell growth by the protein (20, 21). In aggregate, these studies support the existence of the following signal transduction pathway: PKA-LKB1-AMPK-tuberin-Rheb-mTOR, in which PKA activates LKB1, which in turn activates AMPK. AMPK then activates tuberin, which promotes GTP hydrolysis and the inactivation of Rheb, thereby inhibiting mTOR.

The results of the present study provide further support for
The finding that perfusion with 4× amino acids promotes AMPK phosphorylation on Thr-172 in the absence of glucagon presents at least two ambiguities. First, although a 4× concentration of amino acids induces AMPK(Thr-172) phosphorylation, LKB1 phosphorylation on Ser-428 is unaffected. This result would suggest the existence of another kinase that can phosphorylate AMPK on Thr-172, that LKB1 activity can be regulated through mechanisms other than phosphorylation of Ser-428, or that amino acids repress an AMPK(Thr-172) phosphatase. In this regard, at least eight phosphorylation sites have been identified on LKB1 (39). Whether amino acids may regulate the kinase through phosphorylation of one or more of those sites is unknown. We also considered whether an amino acid-induced increase in protein synthesis may decrease the hepatic ATP:AMP ratio. However, no change in the ATP:AMP ratio was detected by high pressure liquid chromatography analysis of extracts of livers perfused in the presence of 1× compared with 4× amino acid (data not shown). Furthermore, we argue that the activation of AMPK represses signaling through mTOR, yet a 4× amino acid concentration promotes phosphorylation of both AMPK and the mTOR targets 4E-BP1 and S6K1. In fact, the relative phosphorylation of AMPK in livers perfused in the presence of 4× amino acids in the absence of glucagon is similar to livers perfused in the presence of 1× amino acids and glucagon. A possible explanation for this apparent inconsistency is that although amino acids promote phosphorylation of Thr-172 on AMPK, they also act downstream of AMPK to enhance signaling through mTOR. In this model, the stimulatory effect on mTOR would be dominant to the inhibitory effect caused by activation of AMPK. In support of this idea, the combination of glucagon and amino acids enhances AMPK phosphorylation to a greater extent than amino acids alone and therefore may overcome the stimulatory effect of amino acids on mTOR signaling. Regardless of the mechanism involved, the results suggest that a delicate balance exists between the inhibitory effects of AMPK and the positive effects of amino acids on signaling through mTOR.

As noted above, the results of the present study suggest that glucagon represses amino acid-induced signaling through mTOR. However, although glucagon represses the amino acid-induced phosphorylation of the mTOR substrate S6K1, phosphorylation of two proteins downstream of S6K1, rpS6 and eIF4B, was enhanced in livers perfused with glucagon, independent of amino acid concentration. Moreover, the glucagon-induced increase in rpS6 phosphorylation occurred only on Ser-235/Ser-236, not Ser-240/Ser-244. A recent study (48) reported that the phosphorylation of rpS6 on Ser-235/Ser-236 is enhanced in cells lacking both S6K1 and S6K2 treated with insulin and epidermal growth factor, even in the presence of the mTOR inhibitor rapamycin. Moreover, in that study, p90rsk may phosphorylate rpS6 on Ser-235/Ser-236 but not Ser-240/Ser-244. In contrast, the hormones had no effect on rpS6 on Ser-235/Ser-236. The results of the present study agree with the conclusion that p90rsk may phosphorylate rpS6 on Ser-235/Ser-236 but not Ser-240/Ser-244 and further suggest that the kinase may also phosphorylate eIF4B.

The functional consequences of the interaction between glucagon-induced repression of the mTOR signaling pathway and the simultaneous activation of the ERK pathway are likely to be complex and will require further studies for elucidation. For example, although amino acid-induced phosphorylation of both 4E-BP1 and S6K1 was repressed by glucagon, the hormone promoted phosphorylation of two targets that are considered traditionally to be downstream of S6K1, rpS6 and eIF4B.

The finding that perfusion with 4× amino acids promotes AMPK phosphorylation on Thr-172 in the absence of glucagon redistributes into mTOR signaling, yet the phosphorylation of both AMPK and the mTOR targets 4E-BP1 and S6K1 is repressed by glucagon, the hormone had no significant effect on phosphorylation of S6K1 at the lower amino acid concentration. The lack of effect on S6K1 may be because the protein is already mostly dephosphorylated, and thus a decrease in the phosphorylation state would be difficult to detect. Because more than 30% of 4E-BP1 was present in the hyperphosphorylated form in livers perfused with 1× amino acids in the absence of glucagon, a decrease in 4E-BP1 phosphorylation was detected more easily.

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Decreased phosphorylation of 4E-BP1 would be expected to promote the association of the protein with eIF4E, thereby limiting assembly of the active eIF4F complex. Because of its important role in mediating mRNA binding to the 40 S ribosomal subunit, decreased eIF4F availability should lead to a decrease in cap-dependent mRNA translation. In contrast, phosphorylation of rpS6 is reported to be associated with enhanced translation of mRNAs containing a terminal oligopyrimidine tract adjacent to the 5′-cap structure (reviewed in Refs. 49 and 50). Thus, enhanced phosphorylation of rpS6 induced by glucagon may cause a preferential increase in the translation of terminal oligopyrimidine mRNAs. However, the role of Ser-235/Ser-236 compared with Ser-240/Ser-244 in the regulation of cap structure (reviewed in Refs. 238/Ser-236 compared with Ser-240/Ser-244 in the regulation of cap structure (reviewed in Refs. 239/Ser-236 compared with Ser-240/Ser-244 in the regulation of cap structure (reviewed in Refs. 23)

Overall, the results of the present study demonstrate that glucagon represses amino acid signaling through mTOR and suggest that the mechanism for this effect involves activation of eIF4B in altering its function is relatively unexplored. 

Acknowledgment—We thank Sharon L. Rannels for performing the liver perfusions.
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J. Biol. Chem. 2004, 279:54103-54109.
doi: 10.1074/jbc.M410755200 originally published online October 19, 2004

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