REVIEW ARTICLE

Amino-acid-dependent signal transduction

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Recent research carried out in several laboratories has indicated that, in addition to their role as intermediates in many metabolic pathways, amino acids can interact with insulin-dependent signal transduction. In this short review, the current state of this rapidly expanding field is discussed.

Key words: autophagy, insulin, phosphatidylinositol 3-kinase, protein kinase B, S6.

INTRODUCTION

The first indications that amino acids can stimulate signal transduction were obtained in studies on the regulation of hepatic autophagy. In the liver, autophagy is responsible for most of the starvation-induced proteolysis [1]. In response to amino acid deprivation, autophagy is up-regulated to produce amino acids that are needed for survival of the cell and, in the liver, also for gluconeogenesis. Since amino acids are products of autophagy, it is not surprising that they are inhibitors of this process. Hormones that control hepatic autophagy are insulin (inhibitory) and glucagon (stimulatory).

In studies on the mechanism responsible for the inhibition of autophagy by amino acids in isolated rat hepatocytes, we discovered that the addition of amino acids in the absence of insulin or other growth factors resulted in a strong and fairly rapid stimulation ($t_{1/2}$ 10–20 min) of the phosphorylation of ribosomal protein S6 [2,3]. S6 phosphorylation is known to be required for the translation of the terminal oligopyrimidine ('TOP') family of mRNAs that contain an oligopyrimidine tract upstream of their transcription-initiation site. These messengers encode proteins belonging to the protein-translation machinery [4,5]. Maximal stimulation of S6 phosphorylation by amino acids was observed at concentrations similar to those found in the portal vein after feeding. Stimulation of S6 phosphorylation and inhibition of autophagic proteolysis followed the same amino-acid-concentration-dependence and time-dependence. Furthermore, a linear relationship was observed between the extent of S6 phosphorylation and the percentage inhibition of autophagic proteolysis after the addition of amino acids. Moreover, the amino acid specificities of the stimulation of S6 phosphorylation and of the inhibition of proteolysis were identical. Among the various amino acids, a combination of leucine, tyrosine and phenylalanine proved to be particularly effective in stimulating S6 phosphorylation and in inhibiting autophagic proteolysis. Since the stimulation of S6 phosphorylation by amino acids could be completely prevented by rapamycin, it was concluded that mammalian target of rapamycin (mTOR)-dependent activation of 70 kDa S6 kinase (p70^{S6K}) must be responsible for amino-

acid-stimulated S6 phosphorylation [3]. Intriguingly, rapamycin accelerated autophagy in the presence of inhibitory concentrations of amino acids. Acceleration of autophagy by rapamycin under nutrient-rich conditions has also been found in yeast cells [6]. These observations clearly indicate that autophagy, at least in part, is under the control of either mTOR itself or a protein the activity of which is regulated by mTOR in a rapamycin-sensitive manner.

Incubation of hepatocytes with insulin alone neither stimulated S6 phosphorylation nor inhibited autophagic proteolysis unless low concentrations of amino acids were present. Furthermore, amino acids and insulin acted synergistically on both processes [2,3]. At high amino acid concentrations, S6 phosphorylation was maximally induced and could not be further stimulated by insulin. Likewise, in the presence of low, but not of high, amino acid concentrations, addition of glucagon to hepatocytes decreased S6 phosphorylation and stimulated proteolysis.

MECHANISM OF AMINO-ACID-DEPENDENT SIGNALLING

p70^{S6K} and amino acids

Amino-acid-dependent signal transduction and the interaction with insulin-dependent signal transduction appeared not to be unique to hepatocytes and has now been found in various insulin-sensitive cell types. Examples are adipocytes, muscle cells, hepatoma cells, Chinese-hamster ovary (CHO) cells, HT-29 cells and pancreatic β -cells [7–13]. In most studies, phosphorylation (and the activity) of p70^{S6K} was measured and, like S6 phosphorylation in hepatocytes, was found to be stimulated by amino acids in a rapamycin-sensitive manner. Activation of p70^{S6K} requires hierarchical phosphorylation of about ten different sites by independently regulated protein kinases [14,15]. Increases in p70^{S6K} activity by phosphorylation may be due to direct phosphorylation by mTOR of the rapamycin-sensitive site Thr⁴¹², which then promotes the ability of 3-phosphoinositide-dependent protein kinase-1 (PDK1) to phosphorylate Thr²⁵² [16]. (These amino acids correspond to positions 389 and 229 of the short splice variant of p70^{S6K} [17].) As for S6 phosphorylation in hepatocytes [2,3], amino acids and

Abbreviations used: PI 3-kinase, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PDK, phosphoinositide-dependent kinase; PKB, protein kinase B; mTOR, mammalian target of rapamycin; S6, ribosomal protein S6; p70^{S6K}, 70 kDa S6 kinase; eIF-4E, eukaryotic protein-translation initiation factor 4E; 4E-BP1, eIF-4E-binding protein-1; PHAS, phosphorylated heat- and acid-stable protein; PP2A, protein phosphatase 2A; GCN, general control, non-depressible; IRS-1, insulin receptor substrate-1; JAK, Janus kinase; CHO, Chinese-hamster ovary; Leu₆-MAP, a small, cell-impermeant, globular peptide with eight leucine residues on the outside.

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insulin synergized in activating $p70^{S6K}$ in several cell types [7,10,13,18]. Interestingly, the specificity of the amino acid activation was similar to that observed in hepatocytes in that, among the various amino acids, leucine was the most potent stimulator of $p70^{S6K}$ [7,8,10–13,19,20]. However, for full activation of $p70^{S6K}$, other amino acids needed to be present as well. A plausible explanation for the additional role of some other amino acids, which by themselves are not effective in activating $p70^{S6K}$ (e.g. glutamine [9]), might be found in their ability to increase the cell volume, which results from their Na^+ -coupled transport against a concentration gradient. In support of this view, experiments in hepatocytes have indicated that stimulation of S6 phosphorylation by leucine (which does not affect the cell volume [8,21]) can be strongly potentiated by a hypo-osmotically induced increase in cell volume [2,3]. The observation made with Jurkat cells that depletion of glutamine and asparagine in the presence of all other amino acids resulted in inactivation of $p70^{S6K}$ [22] may then be explained by a decrease in cell volume. The mechanism by which changes in cell volume affect signalling is not known at present [but see the subsection 'Summary (Figure 1)' below for a discussion].

Eukaryotic-initiation-factor-4E (eIF-4E)-binding protein-1 (4E-BP1) and amino acids

In addition to $p70^{S6K}$, 4E-BP1 (also known as PHAS-1) is a substrate of mTOR [23–26]. 4E-BP1 has at least five phosphorylation sites that are targets of different protein kinases, and phosphorylation of 4E-BP1 results in the dissociation of 4E-BP1 from the eIF-4E·4E-BP1 complex. eIF-4E (the 7-methyl-guanosine cap-binding protein) then becomes available to form part of the eIF-4F translation-initiation complex, which consists of the scaffold protein eIF-4G, the RNA helicase eIF-4A and the RNA-binding protein eIF-4B [27]. Phosphorylation of 4E-BP1, like that of $p70^{S6K}$, requires the presence of amino acids (leucine in particular [7,9,13]) and amino acids alone, but not insulin alone [7,28], stimulate phosphorylation of this protein in a rapamycin-sensitive manner [7–10,28,28a,29].

Phosphatidylinositol 3-kinase (PI 3-kinase), protein kinase B (PKB) and amino acids

The mechanism responsible for the activation of $p70^{S6K}$ (and also of 4E-BP1) by amino acids and the synergism of their effect with that of insulin has been the subject of intense study. Although in the absence of amino acids insulin alone has no effect on $p70^{S6K}$, the hormone does activate tyrosine phosphorylation of the insulin receptor and of insulin receptor substrate-1 (IRS-1) and increases the activity of PI 3-kinase and PKB [7,10,18,28a], all of which are components upstream of mTOR and $p70^{S6K}$ in the insulin-signalling pathway [30,31]. Conversely, amino acids stimulate phosphorylation and activity of $p70^{S6K}$, but do not stimulate tyrosine phosphorylation of the insulin receptor and of IRS-1 [7–10]. They also do not seem to activate PI 3-kinase [7,10,28a] and PKB [7,8,10,29]. In one study, carried out with hepatocytes, has activation of PI 3-kinase by cell swelling induced by addition of proline or glutamine been reported [32]. Leucine was not tested in that study. In other studies demonstrating activation of $p70^{S6K}$ by insulin alone, proteolytically produced amino acids were shown to contribute to $p70^{S6K}$ activation [28a].

Activation of $p70^{S6K}$ by amino acids is strikingly analogous to that caused by α -adrenergic agents, as recently reported for rat fibroblasts, in that activation of $p70^{S6K}$ also occurred in the absence of activity changes in either PI 3-kinase or PKB [33]. Because amino acids were present at low concentrations in the

culture medium, the possibility that this was due to stimulated transport of amino acids across the plasma membrane was not considered, however.

Notwithstanding the inability of amino acids to stimulate PI 3-kinase activity, as determined after immunoprecipitation of the enzyme from amino-acid-treated cells [7,10], the PI 3-kinase inhibitors wortmannin and LY294002 prevented activation of $p70^{S6K}$ and phosphorylation of S6 [7–10,20,34].

A possible explanation of these apparently conflicting results is that *in vitro* assays of PI 3-kinase do not reflect the true activity of the enzyme in the intact cell because of the existence of several isoforms of the enzyme [35]. Insulin and other growth factors stimulate PI 3-kinase class I, resulting in the production of $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$, which recruit PKB to the plasma membrane and co-localize it with constitutively active PDK1 [31,35]. These phosphatidylinositol phospholipids also induce a conformational change in PKB that allows it to be phosphorylated and activated by PDK1 on Thr³⁰⁸ and Ser⁴⁷³ [31,36]. Class II PI 3-kinase, which has sequence similarity to class I PI 3-kinase within a kinase core region, possesses distinct N- and C-termini with a characteristic C-terminal C2 domain and does not associate with the p85 adaptor protein [35], can also be activated by insulin [37]. However, class II PI 3-kinase has strong preference for $PtdIns$ and $PtdIns4P$ as substrates and does not produce $PtdIns(3,4,5)P_3$ [35]. Class III PI 3-kinase, which is the mammalian homologue of the yeast VPS34, only produces $PtdIns3P$ [35], and its activity may not be affected by insulin, as suggested by the finding that intracellular $PtdIns3P$ levels, in contrast with those of $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$, do not increase in the presence of insulin [38]. As assays of PI 3-kinase have in fact been assays of class I [7,10] PI 3-kinase, the possibility cannot be excluded that amino acids activate class II PI 3-kinase. The observation that autophagy is stimulated by $PtdIns3P$ and inhibited by both $PtdIns(3,4)P_2$ and $PtdIns(3,4,5)P_3$ [39], together with the notion that amino acids are strong inhibitors of autophagy, would be consistent with, but does not prove, activation of either PI 3-kinase class I or class II.

The most likely explanation for the conflicting data concerning the role of PI 3-kinase in amino acid signalling, however, is that the PI 3-kinase inhibitors are not specific and that they also inhibit other signal-transduction components [7,10]. Indeed, direct inhibition of mTOR by wortmannin and LY294002 has been demonstrated, which is explained by the fact that the catalytic domain of mTOR resembles that of PI 3-kinase [40].

A possibility that has not been considered in the literature so far is that the amino acids do not affect PI 3-kinase itself but rather inhibit the activity of the lipid phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), which removes the phosphate group in the 3-position of $PtdIns(3,4,5)P_3$ [41]. Inhibition of PTEN by amino acids would thus result in an increased level of $PtdIns(3,4,5)P_3$ in the absence of activity changes in PI 3-kinase. This would also account for the synergism between the effects of insulin and amino acids. Although attractive, this hypothesis does not explain why amino acids alone do not activate PKB, but do activate $p70^{S6K}$.

mTOR and amino acids

Recent reports indicate that mTOR may be the point where amino acid and insulin signalling converge. Firstly, a rapamycin-resistant $p70^{S6K}$ deletion mutant in CHO cells was also resistant to amino acid depletion and could be activated by insulin alone [7]. Secondly, experiments *in vitro* revealed that PKB could directly phosphorylate Ser²⁴⁴⁸ of mTOR, but that, in intact cells, insulin-dependent, wortmannin-sensitive and rapamycin-insen-

sitive PKB-mediated phosphorylation of Ser²⁴⁴⁸ was dependent on the presence of amino acids [42]. Moreover, in the absence of insulin, amino acids themselves were able to stimulate the phosphorylation of Ser²⁴⁴⁸. Interestingly, PKB could phosphorylate mTOR *in vitro*, but only when mTOR was immunopurified from amino-acid-treated cells [42]. This indicates that amino acids promote the activation of mTOR, either by direct activation of PKB-induced phosphorylation of Ser²⁴⁴⁸ or by inducing phosphorylation of amino acid residues adjacent to Ser²⁴⁴⁸ by an as-yet-unknown protein kinase, which would then facilitate access of PKB [42]. The possibility of an amino-acid-inhibitable protein phosphatase that would be responsible for the dephosphorylation of Ser²⁴⁴⁸ was considered to be less likely, because phosphorylation of Ser²⁴⁴⁸ was not sensitive to known phosphatase inhibitors [42]. Perhaps the proposed unknown protein kinase is also the target of cAMP, which inhibits insulin-mediated phosphorylation and activation of mTOR without affecting PKB [43]. This antagonism between amino acids and cAMP with regard to mTOR activation would nicely explain the opposing effects of amino acids and glucagon on S6 phosphorylation in hepatocytes as discussed above.

Protein phosphatase 2A (PP2A) and amino acids

As the extent of phosphorylation of a particular protein is determined by the balance of phosphorylation and dephosphorylation, it has been proposed that amino acids may inhibit a protein phosphatase acting on p70^{S6K} [7]. Such a mechanism would satisfactorily account for the synergism between amino acids and insulin in stimulating p70^{S6K} activity. Support for this proposal was obtained from cross-linking, co-immunoprecipitation and affinity chromatography experiments showing that p70^{S6K} is tightly associated with protein phosphatase PP2A [44,45], which in mammalian cells is the major soluble serine/threonine phosphatase [36]. Association of PP2A with p70^{S6K} did not occur with the mutant p70^{S6K} mentioned above that is resistant to rapamycin and also to amino-acid-deprivation-mediated dephosphorylation [45]. In intact Jurkat cells, phosphorylation and activation of p70^{S6K} and 4E-BP1 in the absence of amino acids or in the presence of rapamycin could be induced by addition of the PP2A inhibitor calyculin [45]; similar observations were made in HEK-293 cells exposed to hyperosmotic stress [46]. Moreover, treatment with rapamycin resulted in an increase in PP2A activity towards 4E-BP1 as measured *in vitro*. Because mTOR isolated from stimulated cells could phosphorylate PP2A *in vitro*, it was proposed that, in intact cells, phosphorylation of PP2A on Ser/Thr residues by mTOR inhibits its activity [45].

Although this is compelling evidence supporting a role of PP2A in the control of p70^{S6K} activity, the use of PP2A inhibitors has, however, not always yielded unequivocal results. Furthermore, increases in p70^{S6K} phosphorylation, and activation, by PP2A inhibitors is not always found [7,44].

Like p70^{S6K}, PKB is also inactivated by PP2A, and dephosphorylation of PKB induced by hyperosmotic stress can be prevented by calyculin [47]. Whether amino acids are able to inhibit PKB dephosphorylation is unknown at present.

In addition to mTOR, Janus kinase-2 (JAK-2) may be another candidate kinase that phosphorylates PP2A, be it not on serine/threonine residues but on tyrosine residues. PP2A and JAK-2 are closely associated, and phosphorylation of PP2A by insulin-stimulated JAK-2 inhibits its activity. Interestingly, these two proteins were found to be in a complex with PI 3-kinase, and it was proposed that inactivation of PP2A by phosphorylation may

result in amplification of insulin-generated signals at the level of PI 3-kinase [48].

Summary (Figure 1)

From these studies, and in spite of conflicting results, the picture emerges that PP2A acts as a switch that turns off signals generated by insulin (and perhaps other growth factors). Amino acids may interact with insulin signalling, either by inhibiting PP2A activity or by altering the binding of the enzyme to signal-transduction components. If, indeed, PP2A acts at various levels in the signalling pathway, and if the amino acids function as inhibitors of PP2A activity, this would account for the fact that the amino acids alone are so effective as activators of 70^{S6K}, S6 and 4E-BP1 phosphorylation. It would also account for the synergism between amino acids and insulin.

mTOR plays a central role in the amino-acid-sensing mechanism, by analogy to the role of the rapamycin-sensitive TOR proteins in nutrient control in yeast [49–51]. As indicated above, amino acids stimulate the phosphorylation of mTOR, which, in turn, stimulates phosphorylation not only of p70^{S6K} and 4E-BP1, but also of PP2A. This results in inhibition of PP2A, which further enhances phosphorylation of p70^{S6K} and of 4E-BP1. If PP2A would also act on mTOR itself (although there is no direct evidence for this as yet [42]), a feedback loop resulting in autoactivation of mTOR would be set up (Figure 1). However, even if amino acids do not act on a phosphatase with mTOR as a target, but rather on a protein kinase with mTOR as substrate (perhaps by facilitating access of PKB to mTOR [42]), this would still explain why amino acids alone are so effective in stimulating phosphorylation of p70^{S6K} and 4E-BP1. Amino acids would then simultaneously activate phosphorylation and inhibit dephosphorylation of these two proteins. In this context it is important to note that, in yeast, too, the rapamycin-sensitive TOR proteins have been shown to affect PP2A, in this case by modulating its association with a protein called Tap42 [49,50].

If amino acids do, indeed, activate mTOR, by stimulating phosphorylation of either mTOR and/or of PP2A, the question remains by which mechanism amino acids exert these effects. One possibility is that amino acids (e.g. leucine) directly stimulate the protein kinases responsible for these phosphorylations. It is also possible that the plasma membrane contains a separate amino acid receptor connected to an intracellular signalling system which then would have to feed into mTOR. There is some evidence supporting the existence of a specific leucine-binding protein in the plasma membrane which might be such a putative amino acid receptor. Leu₈-MAP (a small, cell-impermeant, globular peptide with eight leucine residues on the outside) binds to this protein and inhibits hepatic autophagic proteolysis [52,53]. However, experiments with this peptide (kindly provided by Dr. G. Miotto, Department of Biological Chemistry, C.N.R. Centro di Studio delle Biomembrane, University of Padova, Padova, Italy) carried out in rat hepatocytes in our laboratory did not show any increase in S6 phosphorylation in hepatocytes after short exposure to Leu₈-MAP. Only after long-term exposure of the cells to this compound (> 1 h) was some phosphorylation of S6 observed, but this may have been attributable to release of free leucine from Leu₈-MAP [54].

Another hypothetical mechanism not involving a specific amino acid receptor in the plasma membrane is a mechanism whereby which cells respond to changes in the extent of aminoacylation of tRNAs, and in which free, uncharged, tRNA in the cell prevents mTOR-mediated p70^{S6K} phosphorylation [29]. This hypothesis is based on observations in yeast where, on amino acid starvation, free tRNA binds with high affinity to the protein

Figure 1 Interactions between amino-acid-dependent signalling and insulin-dependent signalling

Further abbreviations: RVD, regulatory volume decrease; AA, amino acids; X, unknown component; p70S6K, p70^{S6K}; S6-P, phosphorylated S6; PIP2, PtdIns(3,4)P₂; PIP3, PtdIns(3,4,5)P₃; P-Y, phosphotyrosine; PI3K, PI 3-kinase; -S-S-, disulphide bridge.

kinase GCN2 (where GCN stands for general control, non-depressible) and activates this enzyme, because the C-terminus of GCN2 structurally resembles histidyl-tRNA synthetase and other aminoacyl-tRNA synthetases [55]. Activation of GCN2 in yeast subsequently leads to increased synthesis of GCN4, a transcriptional activator of at least 40 genes encoding amino-acid-biosynthetic enzymes. Evidence in favour of free tRNA controlling mTOR-mediated p70^{S6K} activation in mammalian cells was obtained in experiments showing that inhibitors of amino acid-tRNA synthetases counteracted amino-acid-stimulated p70^{S6K} activity. Moreover, inhibition of plasma-membrane amino acid transport also inhibited p70^{S6K} activity, which strongly suggests that amino acids had to enter the cells before they could stimulate p70^{S6K} [29]. If this mechanism is true, the particular potency of leucine in stimulating p70^{S6K} may be explained by the frequency of utilization of this amino acid in protein synthesis and by the existence of multiple leucyl-tRNA synthetases arising from the 6-fold codon degeneracy [7]. The question arises about the identity of the signal-transduction component(s) that would be sensitive to inhibition by tRNA. Because uncharged tRNA did not affect the activity of immunoprecipitated mTOR, it must be postulated that tRNA interacts with an as-yet-unknown signal-transduction component regulating mTOR [29].

Uncharged tRNA as inhibitor of signalling could also explain why an increase in cell volume would potentiate signalling. Increases in cell volume (whether induced by concentrative Na⁺-

dependent amino acid transport or by hypotonicity of the extracellular medium) cause many cell types to respond by releasing KCl in an attempt to restore their original volume, a phenomenon known as 'regulatory volume decrease' [56]. This can result in a considerable decrease in cytosolic chloride concentrations. In hepatocytes, the decrease in chloride concentration following amino-acid-induced cell swelling leads to activation of glycogen synthase phosphatase, and thus to increased glycogen synthesis, because chloride is an inhibitor of this enzyme [57]. Data obtained with *Escherichia coli* enzymes have indicated that, at low concentrations, chloride can also inhibit aminoacyl-tRNA synthetases and can significantly raise the *K_m* value for tRNA of these enzymes [58]. If chloride also inhibits aminoacylation of tRNA in mammalian cells, a decrease in chloride concentration following a regulatory volume decrease would result in a decrease in uncharged tRNA, and hence in activation of signalling. Such a mechanism would also provide a satisfactory explanation for the observation that cell swelling accelerates protein synthesis in hepatocytes [56].

PHYSIOLOGICAL RELEVANCE OF AMINO-ACID-DEPENDENT SIGNALLING

Although most studies on amino-acid-dependent signalling have been carried out with isolated cell systems, there is evidence that amino acids also stimulate signal transduction *in vivo*. Thus the

protein anabolic response after a protein-rich meal in humans [59], mice and rats [60,61] was related to postprandial increases in plasma amino acid concentrations rather than in insulin concentrations; this response was accompanied by an increase in phosphorylation of 4E-BP1 and p70^{S6K} in muscle and liver [60–62]. In another study it was shown that the efficacy of insulin to improve the net nitrogen balance across the human forearm greatly increased when amino acids were infused together with insulin [63].

A fascinating observation is that amino acids not only stimulate signalling in insulin-sensitive cells, but that they also do so in the insulin-producing pancreatic β -cells. In β -cells, amino acids also appear to stimulate phosphorylation of p70^{S6K} and 4E-BP1 in a rapamycin-sensitive and a wortmannin-sensitive manner [13,28]. Amino-acid-dependent signalling in β -cells can occur under conditions when they are unable to secrete insulin. As in other cell types, insulin alone, either added externally or produced by the β -cells upon addition of glucose, is not effective in stimulating p70^{S6K} and 4E-BP1 phosphorylation unless amino acids are also present [13,28]. Again, among the various amino acids, leucine is the most effective [13].

While amino acids will promote β -cell proliferation via increased signalling, recent data indicate that extramitochondrial glutamate in β -cells can also directly enhance exocytosis of insulin, possibly by causing swelling of the insulin-containing granules [64]. Thus, from a point of view of whole-body physiology, these amino acid effects are extremely efficient: amino acids not only increase insulin signalling in insulin-sensitive cells, but they also increase insulin production and excretion from the pancreas.

CONCLUDING REMARKS

The notion that amino acids are not only important as intermediates in metabolism, but can positively interact with insulin-dependent signalling and can also stimulate signal transduction on their own, adds a new element to the control of metabolic pathways. The fact that, in concert with insulin, amino acids can oppositely affect protein synthesis and autophago-lysosomal proteolysis by the same signalling pathway is extremely efficient from the point of view of metabolic regulation. The increase in β -cell function by amino acids further adds to the anabolic function of amino acids.

A corollary of the synergism between amino acids and insulin is that the metabolic effects of insulin not only depend on the concentration of insulin itself (and on the concentrations of counter-regulatory hormones and other factors), but also depend on the ambient amino acid concentration. It may even be speculated that, in certain cases of Type II (non-insulin-dependent) diabetes mellitus, a defect in amino-acid-dependent signalling, rather than in insulin-dependent signalling, may be the underlying mechanism.

Note added in proof (received 28 August 2000)

In a paper published in this journal very recently, Peyrollier et al. [65] reported a 5-fold stimulation of PI 3-kinase by 2 mM leucine in L6 muscle cells. The activation was transient and disappeared after 2 min. PKB activity was not affected by leucine.

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