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Role of amino acids in insulin signaling in adipocytes and their potential to decrease insulin resistance of adipose tissue☆

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Abstract

Recently, our knowledge concerning the role of amino acids in signal transduction in mammals has greatly improved. This significant advance is mainly due to the remarkable discovery that the mammalian target of rapamycin (mTOR) protein kinase, known to be activated in response to a large number of hormones, growth factors and cytokines, is also under the tight control of branched-chain amino acids. Actually, both inputs are necessary to fully activate the mTOR pathway, the main function of which is to increase cell size, via the regulation of translational processes. However, amino acids are able to modulate other biological effects and appear to have unexpected actions, as evidenced by our recent work in rat adipocytes. The aim of this review is to summarize novel findings on the role of mTOR and amino acids in insulin signaling in adipocytes. A possible beneficial impact of the use of amino acids in the treatment of insulin resistance is discussed, and hypotheses about the molecular mechanisms underlying their effect are proposed.

Keywords: Amino acids; PKB/mTOR pathway; Glucose transport; Adipocyte; Insulin resistance

1. Introduction

The emerging literature leaves little doubt that amino acids are acting as signaling molecules and play crucial roles in multiple biological functions. Among these is the regulation of protein metabolism, as well as more unexpected effects such as glucose homeostasis or transcriptional gene regulation. Most of the time amino acids act in synergy with other signaling pathways. For instance, the contribution of amino acids, more precisely the branched-chain amino acids (BCAAs), in the insulin-signaling pathway is clearly demonstrated, but some aspects remain controversial or under debate.

Despite their role in insulin-stimulated protein synthesis, little is known about the implication of amino acids in other biological effects of the hormone, especially in adipose tissue. Our present overview aims at discussing the role of amino acids in insulin signaling in adipocytes with emphasis on their recently revealed role in insulin resistance.

2. Amino acids and the mammalian target of rapamycin pathway in adipocytes

In mammals, the main function of amino acids (especially the BCAAs) is to regulate protein synthesis at the level of translation initiation through the mammalian target of rapamycin (mTOR) pathway [1]. Indeed, mTOR plays a major role in the control of cell growth, by regulating the translational repressor eIF4E binding protein 1 (4E-BP1; also known as PHAS-I) and the ribosomal protein S6 kinase 1 (S6K1; also known as p70S6k) [2]. This process results from the combined action of insulin and amino acids. It is now generally believed that insulin activates mTOR via the PI 3-kinase/protein kinase B (PKB) pathway. Moreover, new intermediates have been recently discovered with the tuberous sclerosis complex and the Ras homolog enriched in brain protein lying downstream of PKB and upstream of mTOR (Fig. 1) [3]. The amino acid pathway is much less characterized, and amino acids have been proposed to activate the mTOR pathway at different levels [4–6]. Moreover, two different laboratories have recently shown

☆ In order to limit the number of references, we have occasionally cited reviews and we apologize to the authors of original papers for not citing them directly.

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that the Class III PI 3-kinase, hVps34, lies upstream of mTOR and is regulated by amino acids (Fig. 1) [7,8].

In adipocytes, the mTOR pathway is involved not only in protein synthesis but also in various biological effects such as leptin secretion and tissue morphogenesis [9]. Moreover, several reports have suggested that mTOR also contributes to the control of glucose uptake. In 3T3-L1 adipocytes, rapamycin increases insulin-stimulated glucose transport, whereas amino acids have the opposite effect [10,11]. However, in the study by Bogan et al. [12] on translocation of GLUT4 in the same cells, both a negative and a positive effect of rapamycin and amino acids, respectively, on insulin-induced GLUT4 translocation were reported. These results appear to be contradictory, but Tremblay et al. have further investigated the effect of rapamycin and failed to observe a change in GLUT4 translocation in the presence of the drug. Rather, they indicate that rapamycin acts via inhibition of insulin receptor substrate (IRS)-1 serine phosphorylation [11], suggesting that the mTOR pathway exerts multiple roles at different levels of the insulin-signaling cascade, resulting either in an acute positive response or, in the longer term, in a negative feedback on glucose transport (mTOR and IRS-1 regulation will be discussed below).

Generally speaking, these observations do not appear to be applicable to freshly isolated rat adipocytes. Indeed, we have tested the effects of amino acids and rapamycin in this model, and there was no significant change in insulin-stimulated glucose uptake [13]. Interestingly, Pham et al. [14] have studied in rat adipocytes the proposed mechanisms regulating the mTOR pathway in different cell lines. Their results led to the conclusion that, at least regarding mTOR, the effects observed in cell lines might not be extended without caution to more "physiological" models, in which cells are resting and therefore have a different behavior.

3. Amino acids in adipocyte insulin signaling and glucose transport

In the course of our study on the role of amino acids in glucose uptake, we took a close look at their impact on insulin signaling. We observed that amino acids inhibit PI 3-kinase activity in response to insulin. This inhibition is likely to be due to mTOR, which exerts a negative feedback loop on insulin signaling by phosphorylating IRS-1 on serine residues, as shown previously in different cell types, including adipocytes [11,15–17]. However, amino acids had no effect on insulin-stimulated PKB (also called Akt) phosphorylation, which correlated with the fact that they did not impair glucose transport [13]. Surprisingly, when the rat adipocytes were treated with wortmannin, a PI 3-kinase inhibitor that robustly decreases insulin-stimulated glucose transport, the addition of amino acids inhibited the effect of the drug. Inhibition of the wortmannin action was also observed with leucine alone, which is known to be, among the BCAAs, the most potent activator of mTOR in adipocytes [18]. Next, we decided to try to determine the target responsible for the restoration of glucose transport when the PI 3-kinase is inhibited, which turned out to be PKB. Indeed, in these conditions, both PKB phosphorylation

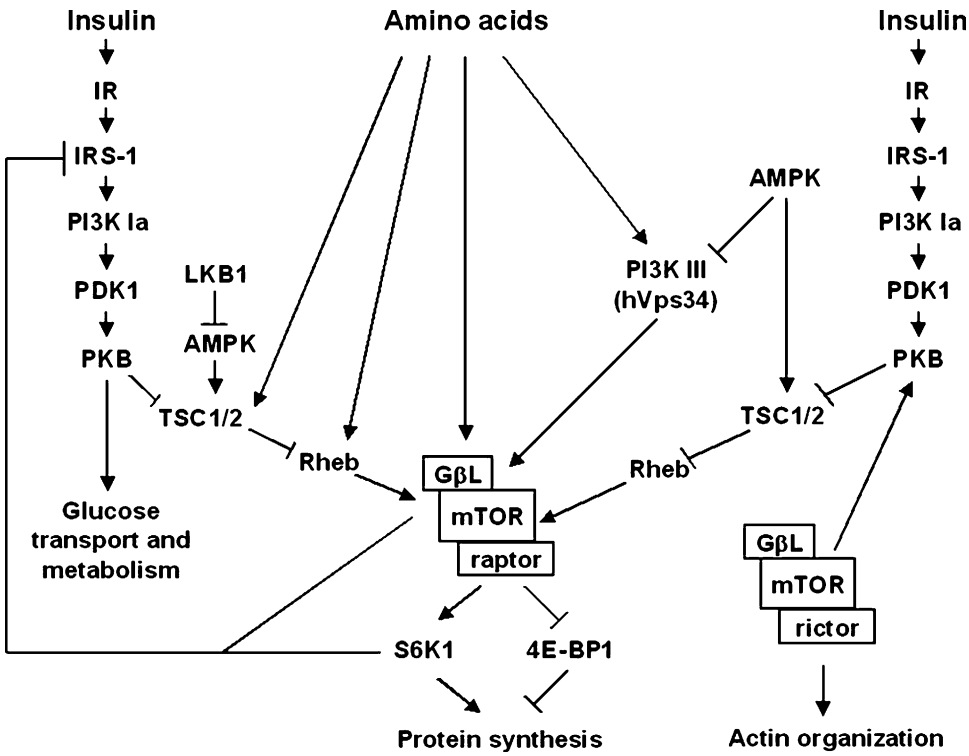

Fig. 1. Regulation of the mTOR pathway by insulin and amino acids.

sites (Thr308 and Ser473) were phosphorylated, and the activity of the β isoform (which is the main activated isoform in response to insulin in rat adipocytes [19]) was partially restored. We have also shown that insulin and amino acids (or leucine) stimulate the mTOR pathway in the presence of wortmannin, as evidenced by the phosphorylation of S6K1 and 4E-BP1 on Thr389 and Thr36/45, respectively [13].

4. Amino acids and the mTOR pathway in insulin resistance

Impairment of insulin signaling largely contributes to the insulin-resistant state observed in Type 2 diabetes and obesity [20,21]. One of the main targets involved in this process is the IRS [22,23]. Together, IRS-1 and IRS-2 account for the activation of the PI 3-kinase/PKB pathway. Thus, by inhibiting their function, most of the downstream metabolic responses induced by insulin are disturbed. It has been shown that several pathways regulate IRS-1 at different levels: (a) increased serine phosphorylation, leading to its dissociation from the insulin receptor or from the regulatory subunit of PI 3-kinase; (b) increased degradation via the proteasome complex; (c) decreased IRS-1 mRNA transcription. The mTOR signaling appears to be involved in these different mechanisms leading to IRS-1 down-regulation. Indeed, mTOR and S6K1 are able to induce IRS-1 serine phosphorylation and affect IRS-1 protein turnover. Furthermore, S6K1 and S6K2 have been shown to be able to reduce IRS-1 gene transcription [24]. In this regard, the mTOR pathway appears to be a negative regulator of insulin action. Interestingly, it has been recently reported that this feedback loop could participate in insulin resistance in obese animal models [25]. These findings suggest that amino acids, ingested in excessive quantity in obesity, lead to overactivation of the mTOR cascade and, therefore, to an increase in IRS-1 inhibition. However, the importance of this regulatory loop in the development and/or the maintenance of insulin resistance remains to be determined.

We have observed different effects of amino acids in this pathophysiological situation, at least in adipocytes. Indeed, we have shown that amino acids have a positive effect on insulin-induced glucose transport in adipocytes of high-fat-fed rats, allowing an insulin response comparable to the one obtained with control rats [26]. Moreover, in adipose tissue explants of *db*/*db* mice, whereas an amino acid mixture has no significant effect, addition of leucine improves insulin-induced PKB activation [13]. Our results show, for the first time to the best of our knowledge, that amino acids could have beneficial effects in pathological situations where the insulin-signaling pathway is impaired, especially at the level of PI 3-kinase. Since we have shown that amino acids in combination with insulin are able to activate PKB independently of PI 3-kinase, we would like to suggest that this pathway, which is "silent" in normal situation, could be important in insulin resistance. Indeed, it is conceivable that this pathway would bypass the classical PI 3-kinase cascade, allowing PKB activation and, hence, restoring insulin glucose transport, at least in part. The fact that both insulin and amino acids are required to obtain the response would explain why it has been overlooked so far and why only particular conditions would permit to reveal its occurrence. Another explanation could be that this effect of amino acids appears to be specific for physiological adipocytes. Indeed, among the different cell types we have tested (including 3T3-L1 adipocytes, rat hepatocytes and intact muscles), the restoration of the PKB/mTOR pathway in the presence of insulin and amino acids (or leucine) when PI 3-kinase is inhibited was obtained only in freshly isolated rat adipocytes and adipose tissue explants [26].

5. Molecular mechanism by which insulin and amino acids allow the activation of the PKB/mTOR pathway in adipocytes with impaired PI 3-kinase activity

In an attempt to determine the proteins involved in this particular effect of amino acids on PKB activation in adipocytes, we studied one of the enzymes responsible for PKB activation, namely, phosphoinositide-dependent protein kinase 1 (PDK1). Indeed, PKB needs to be phosphorylated on Thr308 and Ser473 for full kinase activation, and PDK1 catalyzes Thr308 phosphorylation of PKB [27,28]. Our work led us to conclude that active PDK1 is required to obtain PKB/mTOR pathway activation in the absence of PI 3-kinase activity, suggesting that PDK1 is regulated by

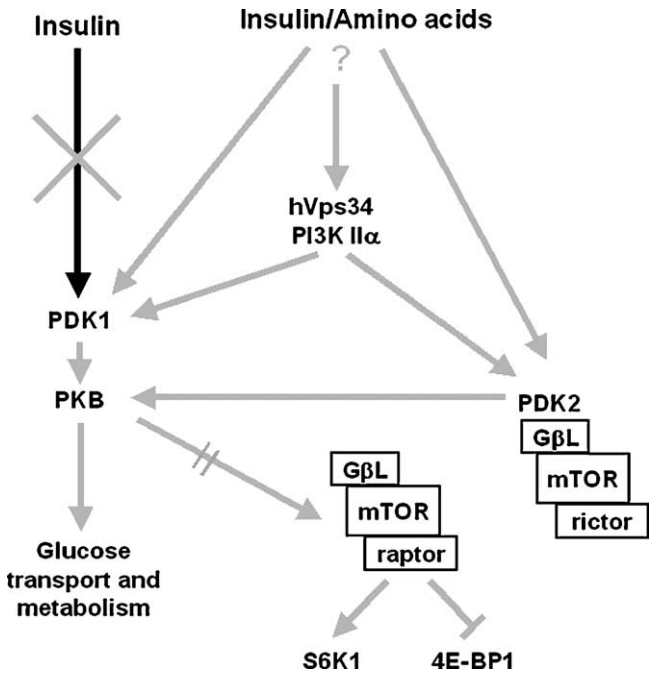

Fig. 2. Stimulatory effect of insulin and amino acids in adipocytes when Class Ia PI 3-kinase is inhibited (by drugs or in pathophysiological situations).

means other than the classical PI 3-kinase-dependent pathway [26]. Interestingly, a similar hypothesis has been proposed by King et al. [29] who have shown PDK1 activation by sphingosine.

While the precise elements of this pathway remain to be identified, we have established that it is rapamycin insensitive [13]. This is an important point, as the negative regulatory loop of insulin signaling implicating mTOR is due to the rapamycin-sensitive complex, comprising the raptor protein. However, we cannot exclude the possible involvement of mTOR, as it is now clear that a rapamycin-insensitive TOR complex is present in mammals [30]. Interestingly, Sarbassov et al. [31] has reported that the rapamycin-insensitive TOR complex formed with rictor is able to phosphorylate PKB on Ser473, corresponding to PDK2. Therefore, it is possible that this complex is involved in PKB activation seen in our conditions. Indeed, our results show that rapamycin fails to inhibit insulin- and amino-acid-induced PKB phosphorylation in the presence of wortmannin (100 nM). Moreover, we found that the effect of insulin and amino acids is lost with higher concentrations of wortmannin (1 μM) [26]. This reinforces the possible involvement of mTOR since Brunn et al. [32] have published that 1 μM of wortmannin irreversibly inhibits mTOR autokinase activity. Additionally, Hresko and Mueckler [33] have shown that the mTOR–rictor complex appears to be the PDK2 in 3T3-L1 adipocytes in response to insulin. Whether this complex is also regulated by amino acids remains to be determined. Thus, when PI3K is inhibited, amino acids and insulin could activate PKB by phosphorylating Thr308 through PDK1 and Ser473 through mTOR–rictor (Fig. 2).

Another possible candidate that could stimulate the PKB/mTOR pathway in the absence of Class Ia PI 3-kinase activation is the Class III PI 3-kinase, hVps34, since this enzyme is able to activate mTOR in the presence of amino acids [7,8]. Insulin has also been shown to activate the α isoform of Class II PI 3-kinase, but its precise role remains unknown [34]. In conclusion, it is conceivable that amino acids and insulin could restore the PKB/mTOR pathway when the Class Ia PI 3-kinase is inhibited and/or in insulin-resistant adipocytes through other classes of PI 3-kinases (Fig. 2). Since these lipid kinases are poorly characterized, it appears to be urgent to further understand their roles in the insulin- and amino-acid-signaling pathways and especially to determine if, in pathophysiological situations, they could take over from Class Ia PI 3-kinase when this one is defective.

In conclusion, we have revealed a novel role of amino acids in insulin signaling and, more precisely, a positive effect of amino acids (and leucine) in the setting of insulin resistance. This pathway could play an important role by replacing the classical PI 3-kinase cascade, usually impaired in insulin resistance, in order to restore downstream signaling and glucose transport. This combined effect of insulin and amino acids appears to be specific for adipose tissue and does not require the rapamycin-sensitive TOR complex (the latter being more implicated in the negative regulation of insulin action). Rather, it may involve other classes of PI 3-kinases and the rapamycin-insensitive TOR complex.

We want to emphasize the concept that, although amino acids induce multiple biological responses, presumably resulting from elaborate nutrient regulation, the ultimate outcome is most likely dependent upon both tissue type and the physiological and/or pathophysiological setting. For example, amino acids appear to display opposite roles in glucose homeostasis in adipocytes. Thus, it is important to try to determine which effect will prevail in any given situation. This is likely a very difficult challenge, but it is important to determine whether amino acids could be useful in the treatment of insulin resistance to improve the defects of glucose metabolism.

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