Amino acid- and lipid-induced insulin resistance in rat heart: molecular mechanisms

Ileana Terruzzi a,*, Sonia Allibardi b, Paola Bendinelli c, Paola Maroni c, Roberta Piccoletti c, Flavio Vescò a, Michele Samaja b, Livio Luzi d,a

a Dipartimento di Medicina, San Raffaele Scientific Institute, Università degli Studi di Milano, Milan, Italy
b Dipartimento di Scienze Mediche, Università degli Studi di Milano, Milan, Italy
c Istituto di Patologia Generale and Centro di Studio sulla Patologia Cellulare del CNR, Università degli Studi di Milano, Milan, Italy
d Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Milan, Italy

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Abstract

Lipids compete with glucose for utilization by the myocardium. Amino acids are an important energetic substrate in the heart but it is unknown whether they reduce glucose disposal. The molecular mechanisms by which lipids and amino acids impair insulin-mediated glucose disposal in the myocardium are unknown. We evaluated the effect of lipids and amino acids on the insulin stimulated glucose uptake in the isolated rat heart and explored the involved target proteins. The hearts were perfused with 16 mM glucose alone or with 6% lipid or 10% amino acid solutions at the rate of 15 ml/min. After 1 h of perfusion (basal period), insulin (240 nmol/l) was added and maintained for an additional hour. Both lipids and amino acids blocked the insulin effect on glucose uptake (P<0.01) and reduced the activity of the IRSs/PI 3-kinase/Akt/GSK3 axis leading to the activation of glucose transport and glycogen synthesis. Amino acids, but not lipids, increased the activity of the p70 S6 kinase leading to the stimulation of protein synthesis. Amino acids induce myocardial insulin resistance recruiting the same molecular mechanisms as lipids. Amino acids retain an insulin-like stimulatory effect on p70 S6 kinase, which is independent from the PI 3-Kinase downstream effectors. © 2002 Published by Elsevier Science Ireland Ltd.

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1. Introduction

The heart is one of the most metabolically active tissues in the body, due to its continuing mechanical workload (Frayn, 1983). For its energy needs, the heart extracts free-fatty acids (FFA), glucose, lactate, pyruvate and ketone bodies from the systemic circulation (Taegtmeyer, 1985) but only the contribution of FFA and glucose was extensively studied. In normal conditions the heart preferentially oxidizes FFA substrates for energy production (Lassers et al., 1972; Rogers et al., 1977). A competition between glucose and FFA shifts myocardial metabolism towards the second substrate, showing the ability of FFA to stimulate lipid oxidation and to reduce glucose oxidation (Van Der Vusse and De Groot, 1992; Randle, 1998). This competition is regulated according to a general mechanism described in isolated rat heart as a cycle (the glucose-fatty acid cycle) by Randle (Randle et al., 1963). Randle also postulated that FFA impair insulin stimulated glucose uptake in skeletal muscle trough the inhibition of pyruvate dehydrogenase, which increases glucose-6-phosphate levels and consequently inhibits hexokinase activity (Randle et al., 1994). Evidence for this mechanism in the skeletal muscle was provided by Boden (Boden et al., 1991, 1994), who found an increased glucose-6-phosphate level at plasma FFA concentration ≥0.75 mmol/l and suggested a decrease in glycogen synthase activity. More recent studies related the FFA-induced insulin resistance to a defective glucose transport/phosphorylation activity (Roden et al., 1996; Dresner et al., 1999;
Griffin et al., 1999). The mechanisms responsible for the impaired insulin action in the presence of high circulating FFA levels are not yet completely understood, but they may include changes in the activity of important components of the insulin signaling cascade affecting glucose metabolism.

Amino acids, like FFA, can be used as energy substrate by certain tissues. Amino acids not only stimulate protein synthesis and inhibit proteolysis (Castellino et al., 1987; Tessari et al., 1996; Kimball et al., 1996) but they also reduce glucose uptake in overnight fasted humans (Ferrannini et al., 1988). In the forearm (Flakoll et al., 1992) amino acids reduce the sensitivity and the maximal responsiveness to insulin action on glucose uptake. In vitro studies proved that amino acids reduce the glucose uptake in the skeletal muscle (Goldberg and Odyssey, 1972; Buse et al., 1972). Therefore, we hypothesized that amino acids may reduce the insulin-stimulated glucose uptake by a substrate competition similar to that described for FFA.

These findings prompted us to investigate the cellular mechanisms by which amino acids modulate insulin action along the insulin signal transduction pathway. Amino acids act in a bi-directional manner: they activate important steps for protein synthesis (p70S6 kinase and PHAS-1) and at the same time they inhibit intermediates important for glucose metabolism, including IRS and PI3-kinase (Patti et al., 1998). Both amino acids and FFA concentrations are increased in poorly controlled diabetes mellitus (Luzi et al., 1990), possibly leading to an impairment of glucose utilization by the heart. Aim of this study was to evaluate the effect of high circulating lipids or amino acids levels (as found in poorly-controlled diabetes mellitus) on the myocardial insulin-stimulated glucose uptake and to explore the target proteins potentially involved in such modulatory effects. The present results show that both lipids and amino acids impair the insulin-induced heart glucose uptake and modulate the post-receptor insulin-signaling cascade in rat heart.

2. Materials and methods

2.1. Materials

Anti-PI 3-kinase p85 rabbit antiserum, anti-phospho-Ser21 glycogen synthase kinase 3 alpha (GSK3α), and anti-IRS2 polyclonal antibodies were purchased from Upstate Biotechnology Inc. (Lake Placid, NY, U.S.A.). Anti-phospho-Akt (Ser473) polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphotyrosine antibody cross-linked to horseradish peroxidase (RC20-HRP) was purchased from Transduction Laboratories (Lexington, KY, USA). Enhanced Chemiluminescence (ECL) Western-blotting detection kit and [γ-32P]ATP (3000 Ci/mmol) were purchased from Amer sham International (Bucks, UK). All other reagents were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

2.2. Isolation and perfusion of rat heart

Sprague–Dawley Charles River outbred rats (250–280 g) were anaesthetized by i.p. injection of heparinized sodium thiopental and the heart was isolated and mounted onto the Langendorff apparatus system (Samaja et al., 1999). The perfusing buffer (KH2PO4 1.2 mM, NaCl 144.1 mM, KCl 4.7 mM, Na2SO4 1.2 mM, Na2EDTA 0.5 mM, MgCl2 1.2 mM, CaCl2 3 mM, HEPES 10 mM, pH 7.4, 37 °C) contained glucose (16.6 mM) as exogenous energy substrate. The perfusate was oxygenated (100% O2) in a membrane oxygenator and circulated at the rate of 15 ml/min by a roller pump (mod. 503 S, Watson–Marlow, Falmouth, Cornwall). After heart stabilization (20 min) 80 ml of the buffer were recirculated through the system. The duration of the experiments was 120 min. The heart performance was monitored during the whole time-course of the experiment by recording the coronary pressure by a pressure transducer (Harvard Apparatus model 52-9966, Natick, MA) inserted between the pump and the heart.

2.3. Experimental protocol

Three independent experiments were performed, and each of them was performed either in the presence or in the absence of insulin. One group of five isolated hearts received perfusate containing glucose alone (GLC). Other two groups of five hearts each received an additional energy substrate represented by 10% amino acids solution (Freamine III 8.5%, Baxter, Deerfield, IL) (GLC + AA) and 6% lipids solution (Intralipid 10%, Fresenius Kabi, plus Heparin 5.000 UI) (GLC + LIP), respectively. In all experiments after the first hour of study (basal period) insulin (Actrapid 240 nmol/l, Novo Nordisk, Sweden) was added directly to the perfusate. During the experiment the glucose concentration in the perfusate was measured every 30 min by an enzymatic method (Ripamonti et al., 1984) in order to calculate the rate of glucose uptake. At the end of perfusion (120 min), hearts were freeze-clamped with Wollenberger clamps cooled to the temperature of liquid nitrogen. The 1 h insulin stimulatory period was chosen since is a reasonable time for glucose uptake to reach a steady-state under hyperinsulinaemic conditions. For each group of experiments hearts used as control were...
perfused as described, without adding insulin to the perfusate to evaluate the effect of amino acids and lipids on the activity of the kinases involved in the insulin signaling pathway. Three replicates for each group without insulin were performed.

2.4. PI 3-kinase immunocomplex kinase assay

The tissue was homogenized in lysis buffer containing: 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.75% Triton X-100, 50 mM β-glycerophosphate, 2 mM sodium orthovanadate (Na₃VO₄), 1 mM MgCl₂, 1 mM CaCl₂, 100 mM NaF, 2 mM EDTA, 1 mM PMSF, 10% glycerol, 10 μg/ml aprotinin, 10 μg/ml leupeptin. After 1h on ice, detergent-insoluble material was removed by centrifugation at 12 500 g for 45 min and 300 μg of lysate proteins were preclarified with normal rabbit serum and protein A-Sepharose. The supernatants were incubated with 1.5 μl anti p85 antiseraum for 2 h at 4 °C and the immunocomplexes were collected with protein A-Sepharose. After extensive washing, the immunoprecipitates were incubated in a reaction mixture containing: 12 mM MgCl₂, 50 μM ATP, 10 μg of sonicated phosphatidylinositol and 10 μCi of [γ-32P]ATP (Folli et al., 1992). The kinase reactions were carried out for 10 min at 30 °C, stopped by addition of 20 μl of 8 M HCl, 160 μl of a 1:1 mixture of chloroform:methanol and 20 μg phosphatidylinositol 4-phosphate (PI4-P) as standard. Extracted phospholipids were applied to silica gel thin layer chromatography (TLC) plates coated with 1% potassium oxalate. TLC plates were developed in chloroform:methanol:water-ammonia (60:47:11.3:2, v/v), subjected to autoradiography and iodine vapors (Cartwright, 1993). Phosphorylated PI3-P was identified by co-migration with PI4-P and quantified by Instant Imager from Packard.

2.5. Immunoblot analysis of phosphorylated GSK3, phosphorylated Akt and p70 S6 kinase

The tissue was homogenized in buffer containing: 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EGTA, 80 mM β-glycerophosphate, 1 mM PMSF, 2 mM DTT, 1 mM Na₃VO₄, 0.1 mM sodium molybdate, 0.1% Triton X-100, 10 μg/ml aprotinin, 10 μg/ml leupeptin (Piccoletti et al., 1994). After 30 min on ice the samples were centrifuged at 100 000 x g for 60 min and 200 μg of supernatant proteins were subjected to immunoprecipitation with preimmune rabbit serum (mock precipitation) or with 2 μg of anti-ERK1 and anti-ERK2 antibodies and protein A-Sepharose. Immunoprecipitates were washed extensively and then incubated at 30 °C for 10 min in 100 μl of kinase buffer containing: 25 mM Tris–HCl pH 7.4, 1 mM DTT; 25 mM β-glycerophosphate, 10 mM MgCl₂, 50 μM ATP, 25 μg of myelin basic protein (MBP), protease inhibitors and 10 μCi of [γ-32P]ATP. The beads were spun, aliquots of 20 μl were spotted onto P-81 filters and counted in a liquid-scintillation counter. Mock precipitate counts were subtracted from ERK immunoprecipitate counts.

2.7. Immunoblot analysis of IRS1 and IRS2

The tissue was homogenized (1:10 w/v) in boiling lysis buffer containing 62.5 mM Tris–HCl, pH 6.8; 2% SDS; 10% glycerol; 2 mM Na₃VO₄; 40 mM β-glycerophosphate; 10 μg/ml leupeptin and 10 μg/ml aprotinin. Homogenates were boiled for 5 min at 100 °C and centrifuged at 8000 x g for 10 min at 4 °C. To study IRS1 and IRS2 phosphorylation, 100 μg of solubilized myocardial proteins were separated by 7% SDS-PAGE and transferred to PVDF membranes. Blots were
blocked in 1% BSA in TTBS overnight at 4 °C and then probed with monoclonal anti-phosphotyrosine antibody RC20-HRP (1:2000) for 1 h at room temperature. After several washes in TTBS, immunoreactive bands were detected by ECL method. To identify IRS1 and IRS2 proteins, identical samples on the same blot were analyzed with anti-IRS1 and anti-IRS2 antibodies, following the protocols described above for p70 S6 kinase and phospho-GSK3, respectively. Alternatively, identification of IRS1 and IRS2 was performed after stripping the anti-phosphotyrosine antibodies (in 2% SDS, 100 mM β-mercaptoethanol, 62.5 mM Tris pH 6.7 for 30 min at 50 °C) and reprobing the same blot with anti-IRS1 and anti-IRS2 antibodies.

2.8. Other procedures

The protein content was estimated by Bradford’s method (Bradford, 1976) or with a detergent-compatible protein assay (Bio-Rad, Richmond, CA, USA). Quantitative measurement of immunoreactive bands was performed by densitometric analysis using IMAGE MAST-ER Software (Pharmacia, Milwaukee, WI, USA).

2.9. Statistical analysis

Mean±S.E.M. of all measurements were compared among groups by ANOVA. The changes from basal within each group were analyzed by Student’s t-test for paired data.

3. Results

The heart rate and the coronary perfusion pressure were continuously monitored throughout the experiment. Both parameters were comparable among the groups and did not change with time (data not shown).

Myocardial glucose uptake in the three experimental conditions is shown in Fig. 1. When glucose alone was supplied, insulin administration doubled the myocardial glucose uptake with respect to basal (P < 0.05). When amino acids or lipids were supplied together with glucose, the basal glucose uptake was similar to glucose alone but insulin was no more able to stimulate glucose uptake. These results show that both energy substrates suppress insulin action on glucose utilization (P < 0.01 versus glucose alone).

Fig. 2 shows the activity of PI 3-kinase, an upstream component in the pathway of insulin stimulated glucose transport (Cheatham et al., 1994). To evaluate the PI 3-kinase activity, solubilized proteins from myocardial tissues were subjected to immunoprecipitation with antibodies directed against the p85 regulatory subunit of PI 3-kinase and PI 3-kinase activity was assayed by measuring the transfer of 32P from [γ-32P]ATP to the 3-position of PI. The figure shows the histogram of the pmoles of 32P incorporated into PI3-P. Myocardial PI 3-kinase activity after heart perfusion with nutrients is reported as open bars when in the absence of insulin, and as closed bars when in the presence of insulin.

In the absence of insulin, PI 3-kinase activity was similar among groups. When insulin was infused, PI 3-kinase activity was markedly stimulated in all groups. However, amino acids and lipids reduced the insulin stimulation of PI 3-kinase activity compared with glucose alone. Fig. 7 panel A, shows an autoradiograph of TLC plate obtained in a typical experiment with insulin.

Fig. 3 shows the phosphorylation of serine/threonine kinase Akt, a downstream target of PI 3-kinase involved in insulin-signaling pathway (Burgering and Coffer, 1995) leading to glucose utilization and glycogen synthesis (Ueki et al., 1998). We evaluated the phosphorylation status of Akt in myocardial tissue by immunoblotting with a polyclonal antibody, which recognizes Ser 473-phosphorylated Akt. Insulin significantly increased the phosphorylation of Akt in all groups. However, amino acids and lipids reduced the insulin-induced Akt phosphorylation compared with glucose alone. Fig. 7, panel B, shows an autoradiograph of an immunoblot obtained in a typical experiment with insulin.

Fig. 4 shows the phosphorylation of GSK3, the major substrate of Akt from which depends its phosphorylation after insulin stimulation (Cross et al., 1995). The phosphorylation status of GSK3 was evaluated by immunoblot analysis of proteins from myocardial tissues using a polyclonal antibody, which recognized Ser 21-phosphorylated GSK3α. We have focused our attention on GSK3α since GSK3β exhibits a higher relative activity toward substrate proteins other than glycogen synthase (Playte et al., 1992). Insulin significantly increased the phosphorylation of GSK3α when glucose alone was infused. The phosphorylation of GSK3α was increased by amino acids and lipids respect to glucose alone in the absence of insulin, but the enzyme phosphorylation could not be further increase by insulin. Therefore, the presence of amino acids and lipids significantly decreased the GSK3α phosphorylation respect to glucose alone. Fig. 7 panel C, shows an autoradiograph of an immunoblot obtained in a typical experiment with insulin.

Since PI 3-kinase/Akt system may play a role in p70 S6 kinase phosphorylation (Burgering and Coffer, 1995) we also studied the behavior of p70 S6 kinase. The activation of p70 S6 kinase is associated with decreased mobility of the protein on SDS-PAGE, indicative of Ser phosphorylation of the enzyme (Long et al., 2000). This electrophoretic behavior may be quantified by densitometric analysis of the unphosphorylated band. Fig. 5
shows that in the absence of insulin, p70 S6 kinase was insignificantly reduced by nutrients. In the presence of insulin amino acids in the perfusion buffer increased the phosphorylation of the enzyme. Lipids did not affect the insulin stimulation of p70 S6 kinase even though we cannot exclude a minor effect of lipids per se in phosphorylating this kinase. Fig. 7 panel D, shows an autoradiograph of an immunoblot obtained in a typical experiment with insulin.

Fig. 6 shows the activity of ERK1 (top panel) and ERK2 (bottom panel), that also play a role in insulin signaling being activated by IRS-dependent and IRS-independent pathways (Zhao and Alkon, 2001). ERK1 and ERK2 activities were assayed in immunocomplex kinase assay using MBP as substrate in the presence of $[^{32}P]ATP$. Insulin increased the MBP phosphorylation in all groups and for both kinases. No effect of amino acids or lipids was evident.

Fig. 8A shows the phosphorylation of the Insulin receptor substrate (IRS) 1 and 2. Solubilized myocardial proteins were resolved by SDS-PAGE and tyrosine
phosphorylated proteins were identified by immunoblotting with anti-phosphotyrosine antibody. Two phosphotyrosine proteins migrating in the range of 165–180 kDa appeared in glucose–insulin perfused myocardial tissue. The phosphotyrosine content of the two protein bands decreased when hearts were perfused with amino acids and lipids respect to glucose alone (Fig. 8A). We hypothesized that the tyrosine phosphorylated proteins represent IRS1 and IRS2 and confirmed their identities by immunoblot analysis using specific antibodies to the carboxy-terminal region of rat IRS1 and IRS2 (Fig. 8B and C). Both IRS1 and IRS2 showed a reduced electrophoretic mobility after perfusion with amino acids and lipids with respect to glucose alone. This electrophoretic behavior could be explained by augmen-

Fig. 4. Amino acids and lipids reduced the insulin stimulation of the phosphorylation of GSK3α. Proteins from myocardial tissues were analyzed by immunoblotting with anti-phospho-Ser GSK3α antibodies. The histogram depicts the means ± S.E.M. of densitometric analysis (OD × mm²) of five independent determinations in the presence of insulin and of three independent determinations in the absence of insulin. Data are expressed as percent of insulin stimulated GSK3α phosphorylation in the presence of glucose alone (taken as 100%). GLC, perfusion with glucose; GLC+AA, perfusion with glucose and amino acids; GLC+LIP, perfusion with glucose and lipids. $, P < 0.01 versus same nutrient without insulin; *, P < 0.01 versus insulin plus GLC alone.

Fig. 5. Amino acids increased the phosphorylation of p70 S6 kinase at the end of the insulin perfusion, whereas lipids had no effect. Proteins from myocardial tissues were analyzed by immunoblotting with anti-p70 S6 kinase antibodies. The histogram depicts the means ± S.E.M. of densitometric analysis (OD × mm²) of five independent determinations in the presence of insulin and of three independent determinations in the absence of insulin. Data are expressed as percent of insulin effect on p70 S6 kinase in the presence of glucose alone (taken as 100%). GLC, perfusion with glucose; GLC+AA, perfusion with glucose and amino acids; GLC+LIP, perfusion with glucose and lipids. $, P < 0.01 versus GLC, *, P < 0.01 versus same nutrient without insulin.

Fig. 6. ERK1 (top panel) and ERK2 (bottom panel) activities. The enzyme activities were assessed by immunocomplex kinase assay using MBP as substrate. The histograms depict the pmoles of ^32P (mean ± S.E.M. of five independent determinations in the presence of insulin and of three independent determinations in the absence of insulin.) transferred to MBP calculated as described under Section 2. Data are expressed as percent of insulin-stimulated ERK activities in the presence of glucose alone (taken as 100%). GLC, perfusion with glucose; GLC+AA, perfusion with glucose and amino acids; GLC+LIP, perfusion with glucose and lipids. $, P < 0.01 versus same nutrient without insulin.
The tyrosine phosphorylation of serine and/or threonine residues was measured in myocardial tissues after 1 h of insulin infusion. The quantitative analysis is reported in Fig. 2. Panel B, Akt phosphorylation. Proteins from myocardial tissues after 1 h of insulin infusion were analyzed by immunoblotting with anti-phospho-Ser Akt antibodies. The quantitative analysis is reported in Fig. 3. Panel C, GSK3α phosphorylation. Proteins from myocardial tissues after 1 h of insulin infusion were analyzed by immunoblotting with anti-phospho-Ser GSK3α antibodies. The upper band is GSK3α, whereas the lower band results from the cross reactivity of the antibody with GSK3β. The quantitative analysis is reported in Fig. 4. Panel D, p70 S6 kinase mobility shift. Proteins from myocardial tissues after 1 h of insulin infusion were analyzed by immunoblotting with anti-p70 S6 kinase antibodies. The densitometric analysis of the lower band is reported in Fig. 5. GLC, perfusion with glucose; GLC+AA, perfusion with glucose and amino acids; GLC+LIP, perfusion with glucose and lipids.

4. Discussion

The purpose of this work was to estimate the regulation of glucose uptake by lipids and amino acids in rat myocardial tissue. Using an isolated rat heart model, we reproduced the results of previous studies showing that lipids induce insulin resistance and we demonstrated for the first time that amino acids also impair insulin action on glucose uptake. We measured glucose uptake by the changes in the perfusate glucose concentration over time. We confirmed our hypothesis that both lipids and amino acids impair myocardial glucose disposal. It is remarkable that this effect was clearly evident despite the near pharmacological dose of insulin employed. Moreover we investigated the possible molecular mechanisms of this substrates-induced insulin-resistance on myocardial glucose uptake. It is well known that the PI-3 kinase/Akt/GSK3 axis regulates the insulin dependent glucose uptake in a variety of tissues. To evaluate the hypothesis that the insulin resistance induced by lipids and amino acids is associated to the down regulation of these kinases, we tested their activation at the end of the insulin infusion period. We found that both substrates share a similar antagonism on the activity of these insulin-stimulated kinases. The relevance of our ex vivo experiments to human pathology, relies on the pathogenic events that high circulating lipids and, possibly, amino acids may determine in common clinical conditions characterized by a defect of nutrient disposal, mainly diabetes mellitus, but also obesity and aging.

PI 3-kinase plays a pivotal role in the mechanisms involved between the cell-surface receptor stimulation and the final insulin mediated responses, including glucose uptake and glycogen synthesis (Shepherd et al., 1996; Holman and Kasuga, 1997). The lipid products of PI 3-kinase represent the messengers responsible of the signal transduction through the steps of insulin-induced signaling cascade regulating carbohydrate metabolism (Nave et al., 1996) and they are involved in mediating downstream events leading to activation of protein kinase B (PKB o Akt) (Stokoe et al., 1997; Alessi et al., 1997, 1998; Stephens et al., 1998). Glycogen synthase kinase-3 (GSK3) is the first identified direct effector of Akt (Welsh and Proud, 1993) from which largely depends its phosphorylation as a consequence of insulin stimulation (Moule et al., 1995; Borthwick et al., 1995; Moule et al., 1997). Such phosphorylation of GSK3 by Akt blocks its ability to phosphorylate its downstream substrate peptide promoting the de-phosphorylation of glycogen synthase (GS) (Alessi and Cohen, 1998) and consequent activation of glycogen synthesis. This insulin dependent inactivation of GSK3 can be blocked by PI 3 kinase inhibitors (Welsh et al., 1994; Cross et al., 1994, 1995; Moule et al., 1995, 1997; Hemmings, 1997) but not by p70 S6 kinase or MAP kinase cascade inhibitors, disproving the involvement of these two pathways in the mechanisms of insulin regulation of glucose metabolism. Thus the inactivation of GSK3 and the consequent activation of GS are the cooperating events by which insulin acts to promote the glycogen synthesis through a PI 3-kinase-dependent mechanism (Moule et al., 1995; Le Marchand-Brustel et al., 1995; Shepherd et al., 1995; Yamamoto-Honda et al., 1995; Sakaue et al.,...
We provided evidence that lipids induce insulin-resistance on glucose uptake. At the same time we found that the insulin-signaling pathway is down-regulated in myocardial muscle. We besides proved that also amino acids are able to induce insulin resistance on glucose uptake and to suppress PI 3-kinase activity, compromising a fundamental transducer of the signaling pathway, which leads to the activation of glycogen synthesis. We speculated that the inhibitory effect of both energy substrates propagates from PI3-Kinase over Akt and GSK3 resulting in a final inability of insulin to stimulate glucose uptake. An important mechanism of insulin-stimulated glucose uptake is through the translocation of glucose transporter 4 (GLUT-4) from intracellular site to the cell surface (James et al., 1989) and there is also evidence that insulin promotes a marked recruitment of GLUT-4 carriers to the plasma membranes in cardiac myocytes (Slot et al., 1991). Different proteins control the glucose-transporter trafficking processes and it has been recently suggested that Akt phosphorylates a component of GLUT-4 vesicles responsible for triggering the translocation of GLUT-4 to the cell surface. The decrement of Akt phosphorylation here documented could have decreased glucose uptake in heart during both lipids and amino acids perfusion by impairing the above mentioned mechanism.

We found a remarkable similarity in the action of amino acids and lipids on PI3-K/Akt/GSK3 pathways that was associated with a similar inhibition of glucose uptake. In contrast there was a striking difference in amino acids and lipids action on p70 S6 kinase, involved in protein synthesis in a variety of cell types including cardiomyocytes (Wang et al., 2000). Previous studies (Pullen et al., 1988; Alessi et al., 1998; Balendran et al., 1999) had proposed that the PI 3-kinase effector (PDK1) controls both the Akt/GSK3 and the p70 S6 kinase pathway. However, in this work we found that amino acids (and not lipids) further activated the insulin stimulated p70 S6 kinase despite they inhibited the insulin stimulated PI 3-kinase. Accordingly, in the absence of insulin, amino acids equally stimulated the p70 S6 kinase without changing PI 3-kinase activity. In agreement with our findings, a temporal analysis of activation of Akt and p70 S6 kinase in L6 muscle cells suggested that their induction could be dissociated and thus insulin could regulate p70 S6 kinase activity by two mechanisms, one PI3K-dependent and one PI3K-independent (Somwar et al., 1998). Mammalian targets of rapamycin, mTor proteins, are a family of kinases that affects the phosphorylation and the activity of proteins regulating mRNA translation, including p70 S6 kinase (Redpath et al., 1996). It has been demonstrated that amino acids availability, per se, regulates this rapamycin sensitive signaling pathway, leading to the activation of p70 S6 kinase through an Akt-independent mechanism in Chinese hamster ovary cells (Wang et al., 1998), as well as in skeletal muscle (Long et al., 2000). We speculate that in the myocardium amino acids activate p70 S6 kinase via mTor dependent mechanism.

ERKs were also investigated as candidate kinases necessary for the stimulation of glycogen synthesis by insulin (Peak et al., 1998). ERK activation is a post-receptor event upon insulin, which could result from IRS/PI3-K pathway (White, 1998) as well as from a distinct pathway involving Shc tyrosine phosphorylation by insulin receptor kinase (Prøn et al., 1994; Bonfini et al., 1996). We used our experimental model to clarify the possible implication of lipids and amino acids in modulation of ERK1 and ERK2 activity. Neither lipids
nor amino acids in presence of insulin were able to modify ERK1 and ERK2 activity respect to glucose–insulin perfusion in myocardial tissue, suggesting that the ERK activating pathway and the PI 3-kinase pathway are independent.

Finally we evaluated the possible modification in IRS phosphorylation. The insulin receptor substrate proteins, IRSs, are the major endogenous substrates of insulin receptor tyrosine kinase. During insulin signaling, IRS protein functions as insulin receptor docking proteins to engage multiple downstream signaling molecules. Both IRS1 and IRS2 are the major insulin-stimulated tyrosine phosphoproteins in muscle cells including cardiomyocytes. There is considerable evidence for a direct role of IRS1 and IRS2 in insulin metabolic action (White, 1998) as well as for suggesting that at least some of insulin resistance forms may depend on IRSs (Whitehead et al., 2000). In addition to tyrosine phosphorylation, IRS1 and IRS2 undergo extensive serine/threonine phosphorylation which cause both a reduced electrophoretic mobility of the two proteins (Giorgino et al., 1993) and conformational changes making them poor substrates for the insulin receptor, contributing to the insulin-resistant state (Paz et al., 1997; Qiao et al., 1999). Recently it has been suggested that the increased serine/threonine phosphorylation of IRS1 in cardiomyocytes of obese rats may play a role in the pathogenesis of the insulin-resistance in these animals (Kolter et al., 1997). We found that amino acids and lipids in presence of insulin decrease both electrophoretic mobility and phosphotyrosine content of IRS1 and IRS2 with respect to glucose alone. Our data suggest that both amino acids and lipids induce IRS serine/threonine hyperphosphorylation and contribute to the attenuation of insulin-dependent PI3-K/Akt/GSK3 pathway.

In conclusion we demonstrated that lipids and amino acids induce insulin-resistance in the myocardium, and may act on the same steps of the insulin-signaling pathway leading to specific effects on glucose metabolism. In contrast, amino acids, but not lipids, share with insulin a similar action on the p70 S6 kinase activation leading to the stimulation of protein synthesis. Our findings lead to a second important research question that needs to be further investigated in order to explain mechanistically the amino acids and lipids interference with the steps of insulin signaling we evidenced. Our data suggest an upstream interference, possibly at the IRS level.

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References


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