Rats with monosodium glutamate-induced obesity and insulin resistance exhibit low expression of $G\alpha_{i2}$ G-protein

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Abstract. In order to test the potential role of inhibitory G-proteins in mechanisms of insulin resistance in adipose tissue of obese animals we determined the content of $G\alpha_{i1}$ and $G\alpha_{i2}$ proteins and an extent of protein tyrosine phosphorylation in epididymal fat tissue cell membranes using immunoblot. Monosodium glutamate-induced obese rats displayed adipose tissue hypertrophy, elevated levels of insulin, leptin and slightly elevated serum glucose. We found significantly decreased protein content of $G\alpha_{i2}$ in adipose tissue plasma membranes of obese rats. This was in accordance with lower protein tyrosine phosphorylation noticed in adipose tissue cell homogenate of glutamate-treated animals. Our results confirm the role of $G\alpha_{i2}$ in development of insulin resistance by crosstalk between the reduced level of inhibitory G-protein and insulin receptor mediated most likely by activation of phosphotyrosine protein dephosphorylation.

Key words: Obesity — Insulin resistance — Glucose transport — Inhibitory G-protein

Introduction

Early postnatal treatment of rats and mice with monosodium glutamate (MSG) leads to the development of obesity and insulin resistance in adult animals (Hirata et al. 1997; Zorad et al. 1997; Macho et al. 2000; de Carvalho Papa et al. 2002). Rats treated with MSG display hypertrophic type of obesity, hyperinsulinemia, hyperleptinemia, reduced serum IGF I, elevated serum cholesterol and triglycerides and decreased insulin-stimulated glucose transport in adipocytes and soleus muscle *in vitro* (Zorad et al. 1997; Macho et al. 2000; Pinterova et al. 2001; Mori et al. 2008). In addition, adipose tissue plasma membranes of MSG-induced obese rats show the presence of defective angiotensin II type I and insulin receptors (Pinterova et al. 2001; Zorad et al. 2003).

The defective insulin receptor and/or impaired GLUT4 glucose transporter translocation can be accounted for insulin resistance at the level of insulin-stimulated glucose transport in adipocytes of MSG-induced obese rats. At basal – non stimulated conditions, only total cell content of GLUT4 protein is reduced in 3-month-old MSG-treated animals (Macho et al. 2000) with no difference in the plasma membrane fraction. This fact directed us to focus our attention toward intracellular pathway of GLUT4 translocation.

It has been shown that inhibitory $G\alpha_{i2}$ G-protein enhances activation and insulin signaling to GLUT4 (Song et al. 2001). The exact mechanism of the linkage between $G\alpha_{i2}$ and GLUT4 translocation is, however, not clear. It is suggested that the inhibitory G-protein-mediated translocation of GLUT4 involves activation of Akt kinase and suppression of phosphatase 1B (Wang et al. 2000; Tao et al. 2001). Earlier studies showed marked decrease in $G\alpha_{i2}$ protein in hepatocyte plasma membranes from diabetic rats (Bushfield et al. 1990). Deficiency of $G\alpha_{i2}$ in adipose tissue and liver impairs insulin action (Moxham and Malbon 1996).

In order to asses the role of inhibitory G-proteins in insulin resistant MSG-induced obese rats we determined the amount of $G\alpha_{i2}$ protein in plasma membranes of adipose tissue. In addition, we studied the extent of protein tyrosine phosphorylation in adipocyte lysate.

Materials and Methods

Male Sprague-Dawley rats (Charles River Wiga, Germany) were injected intraperitoneally with MSG (Sigma, USA), 4 mg/g body weight in the volume not exceeding 0.2 ml,

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every second day during the first 10 days of life. Control rats were injected with 10% NaCl. At the age of 3 months, the animals were decapitated; the epididymal fat tissue was excised, weighted and homogenized as described in Pinterova et al. (2001). Principles of laboratory animal care and all procedures were approved by the Animal Care Committee of the Institute of Experimental Endocrinology of Slovak Academy of Sciences (Bratislava, Slovakia). The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

The epididymal fat was minced by scissors in 50 mmol/l Tris-HCl, 1 mmol/l EDTA, 0.1 μmol/l leupeptin, 1 μmol/l pepstatin, 50 μmol/l phenylmethylsulfonyl fluoride, pH 7.4 buffer with purposely omitted phosphatase inhibitors and homogenized on ice by sonication at low setting. The homogenate was filtered through a nylon mesh and centrifuged at 600 × *g* for 7 min. A part of low-speed supernatant was used for the determination of protein phosphotyrosine content. The second part of the supernatant was further centrifuged at 14,000 × *g* for 15 min; the pellet of crude plasma membranes was obtained and then resuspended in Tris-EDTA buffer. The protein content in both low-speed supernatant and the plasma membranes was determined by the method of Lowry (1951).

For Western blot, 10 and 60 μg protein of solubilized fat tissue total homogenate and plasma membrane fraction, respectively, were separated through 12% polyacrylamide gel electrophoresis and then electrotransferred to Immobilon-P membrane (Millipore, USA). Sigma SDS 7B2 protein standard (Sigma, USA) was used for determination of molecular weight of protein bands.

Immobillon-P membranes with total homogenate proteins were blocked with freshly prepared PBS containing 3% dry milk and 0.2% Tween 20 for 20 min at room temperature. After blocking, the membranes were first incubated with rabbit antiphospotyrosine antibody (Sigma, USA) and after washing with PBS containing 0.05% Tween they were incubated with anti-rabbit IgG antibody conjugated to horseradish peroxidase (Sigma, USA).

The membranes with separated plasma membrane proteins were blocked with PBS containing 3% milk for 20 min at room temperature. This was followed by incubation with rabbit anti-rat antibody against peptide corresponding to amino acids 345-354 of $G\alpha_{i1}$ and 346-355 of $G\alpha_{i2}$ proteins (Anti-G Protein Giα1/2 antibody; Millipore, USA) diluted in freshly prepared PBS-milk at 4°C overnight. After washing with water the membranes were incubated with the secondary anti-rabbit antibody conjugated to horseradish peroxidase. As no optimal housekeeper protein to be applied in the adipose tissue from lean and obese animals is available, we relied on total protein determination.

Protein bands containing immunoreactive phosphotyrosine and the G-proteins were visualized by exposing the membranes to enhanced chemiluminiscence reagent (Amersham Pharmacia Biotech) according to the manufacturer's protocol. The band intensities were quantified by optical densitometry using Kodak DS DC40 camera and 1D Image Analysis Software (Eastman Kodak).

Serum glucose was determined by using auto-analyzer Hitachi 911 (Hitachi, Tokyo, Japan). Serum insulin and leptin were evaluated by using radioimmunoassay kits (Linco Research, USA). The serum was obtained from blood taken from fed animals.

Statistical analysis

The data are expressed as the mean ± SEM for $n = 4–6$ per group. Statistical comparisons were performed using Student's *t*-test.

Results

Adult MSG-treated rats displayed obesity as documented by adiposity index based on the weight of epididymal fat tissue (controls: 1.2 ± 0.2 vs. MSG rats: 2.2 ± 0.2). The obese rats were hyperinsulinemic, hyperleptinemic with moderate hyperglycemia (Tab. 1). Western blot showed markedly decreased protein content of $G\alpha_{i2}$ in adipose tissue plasma membranes of obese animals in comparison to controls (Fig. 1) (2535.8 ± 347.7 vs. 4296 ± 250 arbitrary units, $p < 0.01$, $n = 4$). No difference was noticed in $G\alpha_{i1}$ protein content.

Since fat tissue was homogenized without presence of phosphatase inhibitors, most of the high molecular weight tyrosine phosphorylated proteins usually seen in adipose tissue homogenate (Mooney et al. 1989; Nishimura et al. 1993) was absent on the immunoblot. Phosphotyrosine signal was detected only in two protein bands with molecular weight in range of 58–49 kDa (Fig. 2). The smaller protein was clearly less phosphorylated in obese animals in comparison to controls (2585 ± 474 vs. 5737 ± 689 arbitrary units, $p < 0.01$, $n = 4$).

Table 1. Selected serum parameters

	Controls	MSG rats
Glucose (mmol/l)	5.9 ± 0.1	6.4 ± 0.1 *
Insulin (μU/ml)	33.0 ± 7.9	75.6 ± 10.5 **
Leptin (ng/ml)	17.9 ± 3.1	57.8 ± 2.1 ***

Data are means ± SEM ($n = 6$ for glucose, insulin and leptin). MSG, monosodium glutamate. Significant differences between Controls and MSG rats: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

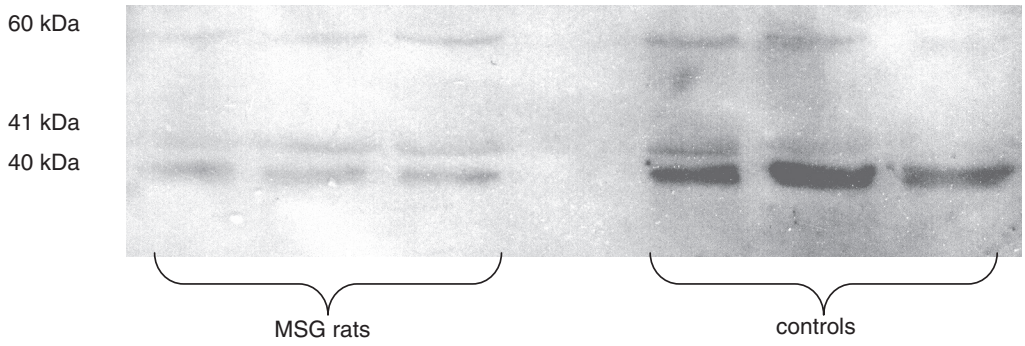

Figure 1. $G\alpha_{i2}$ G-protein immunoblot in plasma membranes of obese (MSG) and control animals. Presented is a representative immunoblot from three separate experiments with identical results. MSG, monosodium glutamate.

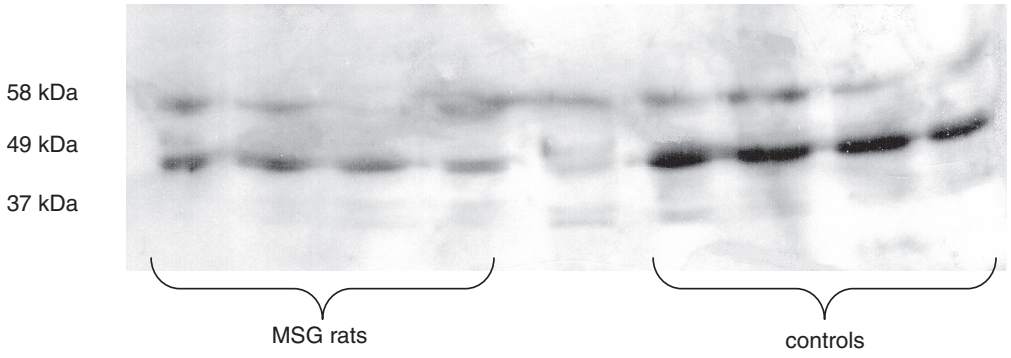

Figure 2. Phosphotyrosine protein immunoblot in adipocyte homogenate from obese (MSG) and control animals. Presented is a representative immunoblot from three separate experiments with identical results. MSG, monosodium glutamate.

Discussion

Our results clearly show reduced amount of $G\alpha_{i2}$ G-protein as well as protein tyrosine phosphorylation in fat tissue plasma membranes of obese animals. We assume that decreased ability of insulin to stimulate glucose transport in adipocytes of MSG-induced obese rats is due to impaired action of $G\alpha_{i2}$ on GLUT4 translocation.

The mechanism of MSG-induced obesity is not quite clear. MSG is causing permanent lesions of hypothalamic arcuate nucleus. This nucleus is producing growth hormone (GH)-releasing hormone (GH-RH). MSG treatment is causing prolonged GH-RH deficiency leading to marked decrease in GH and IGF I serum levels (Maiter et al. 1991; Pinterova et al. 2001). The reduced serum levels of both GH as well as IGF I might be a cause of adipose tissue hypertrophy.

Our previous results showed reduced insulin stimulation of glucose transport and reduced total GLUT4 content in 3-month-old MSG-induced obese animals (Macho et al. 2000). This can be due to the decreased ability of insulin receptors to bind insulin in adipocytes (Zorad et al. 1997, 2003) and/or due to impaired postreceptor pathways. In this study we looked at $G\alpha_{i2}$ G-protein because it seemed to play an important role in insulin-induced GLUT4 translocation (Ferrara and Cushman 1999; Song et al. 2001). In addition, it was shown that *in vitro* $G\alpha_{i2}$ associates with insulin receptor and increases the ability of insulin to activate the receptor autophosphorylation (Kreuzer et al. 2004). Recent data show that removing of negative control caused by regulators of G-protein signaling (RGS proteins) from $G\alpha_{i2}$ results in enhanced insulin sensitivity, resistance to high fat diet induced weight gain and decreased body fat due to increased energy expenditure (Huang et al. 2008). Since MSG-treated animals display opposite features – obesity, insulin resistance and decreased energy expenditure (Yoshioka et al. 1991; Schoelch et al. 2002) to those typical for activated $G\alpha_{i2}$ we checked the amount of the inhibitory G-protein in the adipose tissue. MSG-induced obese rats display a clear reduction in the amount of $G\alpha_{i2}$ protein. We assume that insulin resistance in adipose tissue of MSG-induced obese rats is due to the lowered $G\alpha_{i2}$ activity. In addition, the MSG-induced obese rats, like other animal models of insulin resistance, display

elevated serum free fatty acid levels (Kim et al. 1999). This might be due to reduced antilipolytic effect of insulin as well as lower effectivness of antilipolytic inhibitory G-protein coupled receptors (Langin and Arner 2006) in accordance with our finding of reduced $G\alpha_{i2}$ content in adipose tisssue of obese rats.

Indeed, downregulation of $G\alpha_{i2}$ in liver and adipose tissue using antisense RNA method lead to insulin resistance *in vivo* (Moxham and Malbon 1996). On the contrary, mice expressing constitutively active mutant of $G\alpha_{i2}$ (Tao et al. 2001) in the adipose tissue and muscle exhibit increased insulin sensitivity. Their adipocytes display enhanced GLUT4 translocation probably due to the increased activation of phosphatidyl 3-kinase and Akt (Song et al. 2001). The linkage between $G\alpha_{i2}$ and GLUT4 seems to be the increased protein tyrosine phosphorylation *via* suppression of protein-phosphotyrosine phosphatase 1B (Tao et al. 2001). We measured overall protein-phosphotyrosine phosphatase activity in adipose tissue homogenate by an indirect way determining protein phosphotyrosine content in absence of phosphatase inhibitors. The results show a marked reduction in phosphotyrosine content in protein band with molecular weight below 58 kDa. We did not characterize further this protein in the present study. Nevertheless, we believe that these results clearly demonstrate elevated phosphatase activity in adipose tissue of MSG-induced obese rats. Association of decreased level of $G\alpha_{i2}$ with the decreased protein tyrosine phosphorylation and reduced glucose transport in MSG-treated animals brings additional evidence for suggested important role of $G\alpha_{i2}$ (Huang et al. 2008) in the regulation of adipose tissue metabolism.

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