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Late Effects of Postnatal Administration of Monosodium Glutamate on Insulin Action in Adult Rats

L. MACHO, M. FICKOVÁ, D, JEŽOVÁ, Š. ZÓRAD

Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic

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Summary

Early postnatal administration of monosodium glutamate (MSG) to rats induces obesity, hyperinsulinemia and hyperglycemia in adulthood, thus suggesting the presence of insulin resistance. We therefore investigated the effects of insulin on glucose transport and lipogenesis in adipocytes as well as insulin binding to specific receptors in the liver, skeletal muscle and fat tissues. An increase of plasma insulin, glucose and leptin levels was found in 3-month-old rats treated with MSG during the postnatal period. The attenuation of insulin stimulatory effect on glucose transport was observed in MSG-treated rats. Despite the lower basal and insulin-stimulated glucose uptake, the incorporation of glucose into lipids was significantly higher in MSG-treated rats, suggesting a shift in glucose metabolism towards lipid synthesis in fat tissue. Insulin binding to plasma membranes from the liver, skeletal muscle and adipocytes was decreased in MSG-treated rats. This is in agreement with the lower insulin effect on glucose transport in these animals. Furthermore, a decreased amount of GLUT4 protein was found in adipocytes from MSG-treated obese rats. The results demonstrated an attenuation of insulin effect on glucose transport due to a lower insulin binding and lower content of GLUT4 protein in MSG-treated rats. However, the effect of insulin on lipogenesis was not changed. Our results indicated that early postnatal administration of MSG exerts an important effect on glucose metabolism and insulin action in adipocytes of adult animals.

Key words

Insulin action • Glucose transport • MSG treatment • Rat

Introduction

The early postnatal development of mammals is a period during which the regulation of endocrine gland functions and their relations to the environment are established (Sámel 1968, Hiroshige and Sato 1970, Macho 1975, 1979). At this stage of development, the activity of neuroendocrine regulatory processes is very susceptible to changes of developmental conditions including nutrition, handling or administration of hormones and drugs (Schapiro 1965, Kraus *et al.* 1967, Zarrow *et al.* 1968, Macho 1975, 1979, Csaba 1986). It was repeatedly demonstrated that several interventions during the early postnatal period such as premature weaning, dietary manipulation or hormone administration can modify endocrine functions or metabolic processes later in life (Schapiro 1965, Macho *et al.* 1970, Macho 1979, Sonawane *et al.* 1983, Csaba 1986, Koldovský *et al.* 1995). According to Křeček (1971), these effects were called as"late effects of early postnatal interventions into

Fax +420 2 2492 0590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

development". The administration of monosodium glutamate (MSG) during the early postnatal period is also such an important intervention into the development of experimental animals, because the neurotoxic effects of MSG induce changes in function of endocrine glands and metabolic disturbances observed later in adulthood (Redding *et al.* 1971, Bakke *et al.* 1978, Krieger *et al.* 1979, Nemeroff *et al.* 1981, Dolnikoff *et al.* 1988, Škultétyová *et al.* 1998, Macho *et al.* 1999a,c). The metabolic disturbances are characterized by the development of obesity with lower body mass but with higher percentage of body fat, by normophagia and increased insulinemia with elevation of glycemia. An impaired glucose tolerance was observed in adult rats postnatally treated with MSG, suggesting a diminution of insulin action in target tissues (Hirata *et al.* 1997, Papa *et al.* 1997, Zórad *et al.* 1997). Therefore, the aim of our experiments was to investigate the changes of serum insulin, glucose and leptin levels, and the effects of insulin on glucose transport and lipogenesis in adipocytes of adult rats treated with MSG during the early postnatal period. The binding of insulin to specific receptors in the liver, skeletal muscle and fat tissues and the levels of glucose transporter (GLUT 4) in adipocytes were also determined.

Material and Methods

Male offspring of Sprague-Dawley rats (Charles-River Wiga, Silzfeld, Germany) received an intraperitoneal injections of MSG (4 mg/g of body weight, Merck, Darmstadt, Germany) dissolved in 0.9 % NaCl on alternate days for the first 10 postnatal days. Littermate controls (half of the pups from the same litter) were given an equivalent volume of 10 % saline solution (isosmotic with the MSG solution). The animals were weaned on the 21st postnatal day and housed in standard cages, six animals per cage under controlled conditions (temperature 23±2 °C, 12:12 h light-dark regime). The rats had free access to food and tap water. The animals were used for the experiments at the age of three months. The rats (8 controls and 8 MSG-treated animals) were sacrificed by decapitation after one night of food deprivation and the serum was used for insulin, glucose and leptin determination. In one group of animals, samples of epididymal fat tissue, liver and the quadriceps muscle were immediately removed and frozen in liquid nitrogen. From these tissues, fractions of cell plasma membranes were isolated according to Sakamoto *et al.* (1980) and binding of I^{131}-Tyr_{14}-insulin was determined as described elsewhere (Zórad *et al.* 1985, Macho *et al.* 1999b). The competition curves were analyzed by using the Ligand computer program (Munson and Rodbard 1980), and the capacity of two binding sites was calculated according to De Meyts and Roth (1975).

In other groups of MSG-treated and control rats (8 animals in each group) isolated adipocytes were prepared from adipose tissue immediately after removal of fat pads according to Rodbell (1964). Glucose transport into adipocytes was determined by using 2-deoxy-D-H^3-glucose according to Cherqui *et al.* (1989). All data were corrected for extracellular trapping and passive diffusion by measuring transport in the presence of 0.25 μmol cytochalasine B. The lipogenic activity was assessed by incubation of isolated fat cells for one hour in a Krebs-Ringer-bicabonate solution supplemented with 2 % of albumin, 1 mmol/l of glucose and insulin in the doses of 10^{-11} , 10^{-9} and 10^{-7} mol/l. The incorporation of ^{14}C-U-glucose into total lipids under basal and insulin-stimulated conditions was determined after lipid extraction and expressed in dpm/mg of lipids. Insulin and leptin levels in the serum were determined by radioimmuno-assay (Insulin-Ria kit, NOVO, Nordisk, Denmark, Leptin-Ria kit, Linco Research, St. Louis, Mo, USA), glucose was determined by the orthotoluidine method (Bio-Glu-test, Lachema, Brno, Czech Republic). Proteins in plasma membranes were determined by the method of Lowry *et al.* (1951). Glucose transporter protein GLUT4 was determined by Western blot in fat cell plasma membranes as described in details by Ficková *et al.* (1997).

The significance of differences between control and MSG-treated animals was determined by using the t-test and ANOVA, where appropriate.

Results

The body weight of adult animals treated with MSG during the neonatal period was decreased, while both the absolute and relative weight of epididymal fat tissue was increased due to enlarged fat cell size (Table 1). In agreement with higher weight of fat tissue the serum levels of leptin were elevated in MSG-treated rats (Table 1), thus confirming the accumulation of adipose tissue. The serum levels of insulin were highly increased in MSG-treated animals (Table 1). However, serum glucose concentrations were not decreased, but slightly elevated in comparison to the control group, suggesting the presence of insulin resistance in insulin target tissues.

Table 1. Body mass, absolute and relative weights of epididymal fat pads and liver, serum levels of insulin, glucose and leptin and the content of GLUT4 protein in adipocytes from control rats and rats treated with monosodium glutamate (MSG) during the early neonatal period.

	Control	MSG-treated
Body weight (g)	418±6	335±11**
Epididymal fat (g)	8.06±0.24	11.50±0.35**
Epididymal fat (g/100g bw)	1.9±0.1	3.4±0.2**
Fat cell diameter (μm)	78±2	103±4***
Liver weight (g)	13.6±0.6	10.3±0.5**
Liver weight (g/100g bw)	3.3±0.1	3.1±0.1
Insulin (μU/ml)	31.1±7.5	72.0±10.0**
Glucose (mmol/l)	5.6±0.1	6.1±0.1*
Leptin (ng/ml)	17±3	55±2**
GLUT 4 protein (arb. units)	788±59	340±36*

*Data are means ± SEM. Significant differences between control and MSG-treated rats: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.*

The effect of insulin on glucose transport and on the lipogenesis was therefore investigated in isolated adipocytes. It was observed that basal glucose transport was lower in adipocytes from MSG-treated animals (Fig. 1). A dose-dependent stimulation of glucose uptake was found in adipocytes from control rats (Fig. 1), while the stimulation of glucose uptake by insulin was very low in MSG-treated animals as compared to the controls. The basal incorporation of glucose into lipids was significantly higher in MSG-treated rats (Fig. 2). Insulin stimulated the incorporation of glucose into lipids of isolated adipocytes from both experimental groups in a dose-dependent manner with the exception of the highest insulin concentration in the MSG-treated rats (Fig. 2).

The determination of insulin specific binding in fat cell plasma membranes by the using two-site model revealed a significantly lower binding capacity of high-affinity low-capacity insulin receptor (R1) in MSG-treated rats (Table 2), whereas no differences were noted in low-affinity high-capacity insulin receptors (R2). The decreased amount of insulin-sensitive glucose transporter (GLUT4) in adipocytes was demonstrated in rats with MSG-induced obesity (Table 1).

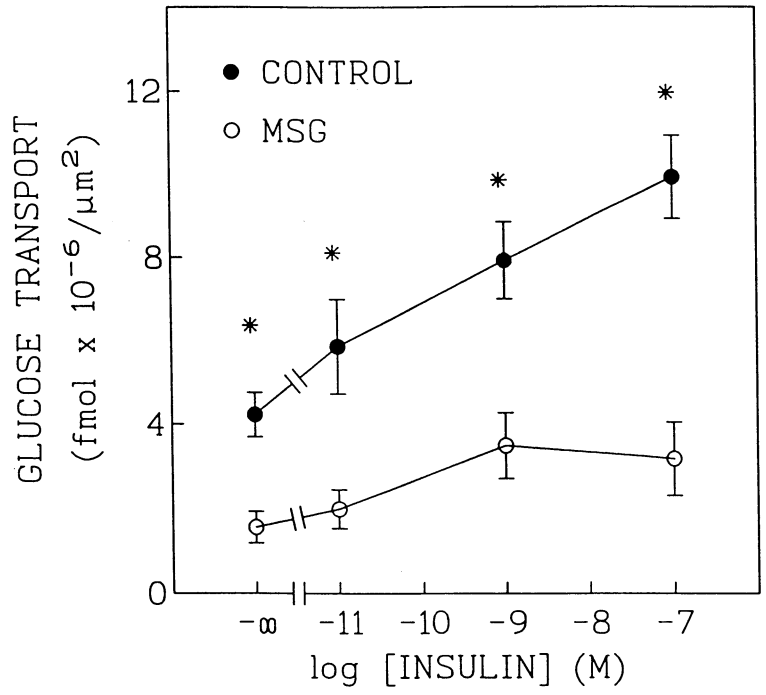

Fig. 1. *Stimulation of glucose transport by insulin in adipocytes from control and MSG-treated rats. The glucose transport is expressed per unit of cell surface. Insulin concentrations in incubation medium were 10^{-11} to 10^{-7} mol/l. Values are means ± SEM, significant difference between control and MSG-treated rats * $p<0.05$.*

The significant decrease of high-affinity low-capacity insulin receptors was found in liver plasma membranes (Table 2) from MSG-treated rats. The low-affinity high-capacity insulin receptors were also decreased in liver plasma membranes from animals neonatally treated with MSG (Table 2).

A highly significant decrease of specific insulin binding and insulin receptors was observed in plasma membranes from skeletal muscles of MSG-treated rats (Table 2), suggesting that besides the adipose tissue, the stimulatory effect of insulin on glucose metabolism was also diminished in muscles.

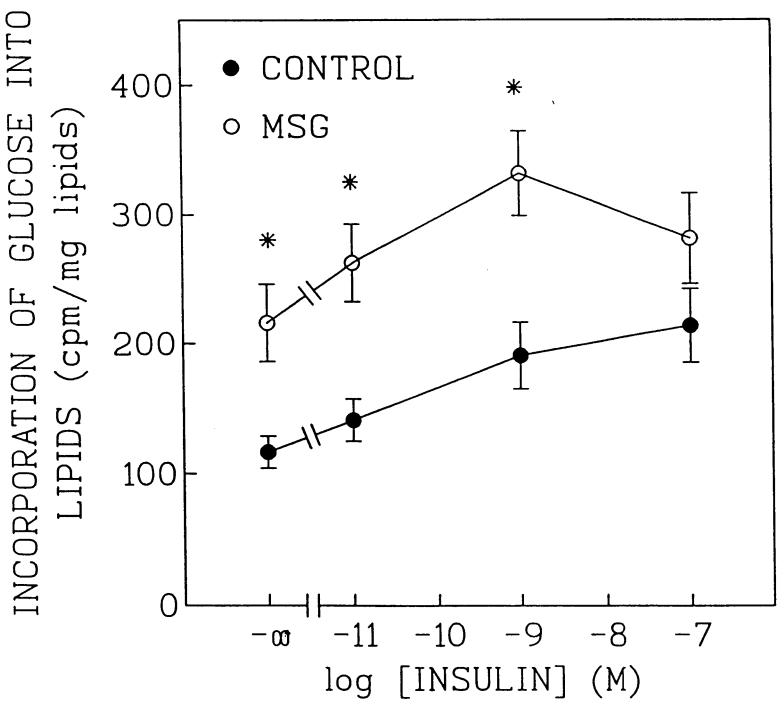

Fig. 2. *Stimulation of lipogenesis from glucose by insulin added to isolated adipocytes from control and MSG-treated rats. Insulin concentrations in incubation medium were 10^{-11} to 10^{-7} mol/l. Values are means ± SEM, significant difference between control and MSG-treated rats * $p<0.05$.*

Table 2. Insulin receptor binding capacity in plasma membrane fractions of liver, adipose tissue and skeletal muscle of control and MSG-treated rats.

Insulin receptors	*Control* R1 (mol 10^{-11}/mg P)	*MSG-treated* R1 (mol 10^{-11}/mg P)	*Control* R2 (mol 10^{-10}/mg P)	*MSG-treated* R2 (mol 10^{-10}/mg P)
Liver tissue	6.08±1.02	3.70±0.48*	32.71±12.60	4.30±0.80*
Fat tissue	5.50±0.74	3.10±0.27**	10.99±5.80	3.65±0.76
Skeletal muscle	0.81±0.11	0.47±0.07*	9.27±0.73	5.60±0.82*

*R1 – high-affinity low-capacity insulin receptors, R2 – low-affinity high-capacity insulin receptors, mg P – miligram of membrane proteins. Data are means ± SEM. Significant differences between control and MSG-treated rats: * $p<0.05$, ** $p<0.01$.*

Discussion

Treatment of neonatal rats with MSG induced several metabolic changes resulting in reduced body and muscle weight but leading to a massive increase of fat tissue content (Remke *et al.* 1988). Hyperinsulinemia and moderate increase of glycemia are present in adult MSG-treated rats (Hirata *et al.* 1997, Zórad *et al.* 1997) in which impaired glucose tolerance and insulin resistance were demonstrated by the intravenous glucose tolerance test and by an insulin-glucose clamp (Hirata *et al.* 1997). The alteration of glucose metabolic pathways (including the mechanism of their development) and the changes of insulin effects in adult MSG-treated rats largely remain unclear (Betrán *et al.* 1992). No such changes in glucose metabolism and endocrine functions were observed when MSG was administered to adult animals, suggesting that the endocrine regulation is very sensitive to MSG only during the early postnatal period.

The insulin resistance observed in obesity induced by MSG treatment could be due to changes in insulin binding or postreceptor insulin effects in target tissues. Our experiments demonstrated a decrease of the stimulatory effect of insulin on glucose transport in adipocytes from MSG-treated rats. This impaired glucose uptake in adipocytes from MSG-treated rats is in agreement with the lower content of glucose transporter (GLUT4) protein in fat cells, as was observed in the

present experiment on rats and in those of Machado *et al.* (1993) and Papa *et al.* (1997) on mice. Besides the fat cells, a significant reduction of GLUT4 protein content was also found in skeletal and cardiac muscles and in brown adipose tissue of mice (Machado *et al.* 1993). On other hand, Marmo *et al.* (1994) did not find any significant differences in glucose transport between control and MSG-treated obese rats aged 3 months. This discrepancy could be due to the long preincubation period of adipocytes used in standard glucose assays performed by these investigators.

The lower stimulatory effect of insulin on glucose transport in adipocytes is also in agreement with the decreased number of insulin receptors in hormone target tissues as was demonstrated in our experiments. The lower values of binding capacity of both high-affinity low-capacity and low-affinity high-capacity insulin receptors were found in adipocytes, skeletal muscles and the liver tissue. The decrease of insulin receptors in MSG-treated rats is probably due to the down-regulation of insulin receptors by hyperinsulinemia and could be responsible for the impaired insulin action on glucose transport in adipocytes. The decrease of insulin binding could also be partially due to the elevated plasma corticosterone concentrations in MSG-treated animals. An inverse relation between plasma corticosterone levels and insulin binding in adipocytes was observed (Olefsky *et al.* 1975, Macho and Ficková 1992). The increased plasma corticosterone levels were repeatedly demonstrated in MSG-treated rats (Dolnikoff *et al.* 1988, Škultétyová *et al.* 1998, Macho *et al.* 1999a,c). The changes of corticosterone levels seem to play an important role in hormonal and metabolic adaptation to fasting (including reduced proteolysis, lipolysis, changes of glycemia, insulinemia and GLUT4 content) of MSG-treated obese rats (Papa *et al.* 1997, Ribeiro *et al.* 1997).

The measurement of lipogenesis in adipocyte showed elevated total glucose incorporation into lipids in MSG-treated rats under basal conditions and in the presence of insulin. However, the increment of glucose incorporation into lipids after the addition of insulin was slightly but not significantly increased in adipocytes of MSG-treated rats as compared to the controls. Such results suggest that despite lower insulin binding and lower insulin stimulation of glucose transport, lipogenesis is elevated in MSG-treated rats. This suggests a shift in glucose metabolism towards lipid synthesis in adipocytes of MSG-treated rats. These results demonstrate that the increase of fat cell size and adipose tissue weight in the MSG-treated group is accompanied by increased glucose metabolism towards lipogenesis. The higher rate of incorporation of radiolabeled substrates (^3H_2O and ^{14}C-glucose) into total lipids was also observed in fragments of epididymal fat pads from MSG-treated rats when it was expressed in relation to the DNA content (Marmo *et al.* 1994).

Our study demonstrated that the stimulatory effect of insulin on glucose transport in adipocytes from MSG-treated rats is diminished. This decrease of the insulin effect is probably due to lower insulin binding and a lower content of the GLUT4 protein. However, the influence of insulin on lipogenesis is not depressed. Despite the lower glucose uptake in adipocytes, basal lipogenesis is increased in MSG- treated rats, suggesting a shift of glucose metabolism to lipid formation in these animals.

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Reprint requests

L. Macho MD, DrSc., Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovak Republic. e-mail: ueenmach@savba.sk