Short Communication

Low Number of Insulin Receptors but High Receptor Protein Content in Adipose Tissue of Rats with Monosodium Glutamate-Induced Obesity

S. Zorad, D. Jezova, L. Szabova, L. Macho and K. Tybitanclova

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

Abstract. In order to better understand the mechanisms leading to insulin resistance, the number of fat tissue insulin receptors, their affinity and insulin receptor protein in rats with monosodium glutamate-induced obesity were studied. Obese rats displayed significantly lower number of insulin receptors with high affinity. Surprisingly, the amount of insulin receptor protein was significantly elevated in these animals. The same relations have been already reported for angiotensin II binding and AT_1 receptor protein in the same model of obesity. Therefore we suggest an existence of general defect of adipocyte cell membrane in monosodium glutamate-induced obesity characterized by the presence of high quantity of impaired receptor protein.

Key words: Insulin receptor — Fat tissue — Adipocyte membrane — MSG rat

Adult mice and rats treated neonatally with monosodium glutamate (MSG) exhibit several endocrine and metabolic abnormalities caused by selective destruction of neurons in the arcuate nuclei of the hypothalamus (Redding et al. 1971). MSG-treated rats (MSG rats) show unchanged or decreased body weight in comparison to control animals, but increased fat tissue mass due to fat cell hypertrophy (Zorad et al. 1997). The MSG rats are further characterized by normophagia, normo-glycemia, hyperinsulinemia, elevated basal corticosterone level, lowered serum IGF-I (Macho et al. 1999; Pinterova et al. 2001) decreased insulin binding as well as decreased insulin-sensitive glucose transport in adipocytes (Macho et al. 2000). In addition, adipocytes of MSG rats display also abnormalities in angiotensin II receptors represented by high amount of receptor protein with profoundly low binding capacity (Pinterova et al. 2001).

A decrease in insulin-stimulated glucose transport in MSG adipocytes can be accounted for lower insulin binding. The question was whether the lower receptor

Correspondence to: Dr. Stefan Zorad, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava 37, Slovakia
E-mail: ueenstef@savba.sk

binding is due to the changes in receptor protein quantity and/or their quality. The purpose of this study was to investigate the relation between number of insulin receptors, their affinity and the receptor protein quantity in MSG-induced obesity.

Male Sprague-Dawley rats (Charles River Wiga, Germany) were injected for the first 10 days of life intraperitoneally with monosodium glutamate (Sigma, USA), 4 mg/g of body weight in the volume not exceeding 0.2 ml. Control rats were injected with 10% NaCl. At the age of 3 months, the animals were decapitated; the epididymal fat tissue was excised and frozen in liquid nitrogen and stored at −75°C until assay. 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, Slovak Republic. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Fat tissue plasma membranes were prepared by differential centrifugation of tissue homogenate in 10 mmol/l Tris-HCl, 250 mmol/l sucrose, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l benzamidine buffer, pH 7.4 (Zorad et al. 2002). Insulin binding was carried out at 4°C for 21 h as described elsewhere (Zorad et al. 2002). Incubation mixture was containing 50 μg of membrane proteins, 0.2 nmol/l mono-^{125}I-(Tyr A14)-insulin (Zorad et al. 1985) and increasing amounts of nonlabeled insulin (10^{-12}–10^{-6} mol/l). The obtained competition data were analyzed with the Ligand program (Munson and Rodbard 1980) using two-binding-site: model based on the existence of high affinity (K1) – low capacity (R1) and low affinity (K2) – high capacity (R2) receptor sites. The amount of membrane proteins was estimated using method of Lowry et al. (1951).

For immunoblot, solubilized fat tissue plasma membranes corresponding to 50 μg of membrane proteins were separated using 12% polyacrylamide gel electrophoresis and then electrotransferred to Hybond membrane. The membrane was blocked in phosphate buffered saline (PBS) containing 3% nonfat dry milk for 60 min. After blotting, the membrane was incubated with rabbit anti-human insulin receptor ß-subunit antibody which cross-reacted with rat insulin receptor (Upstate Biotechnology Incorporation, USA) overnight at 4°C. Then the membrane was washed 1×5 min with PBS containing 3% nonfat dry milk followed by 3×5 min wash with PBS containing 0.05% TWEEN (Sigma, USA). After washing, the second anti-rabbit antibody linked to horseradish peroxidase (Calbiochem, Germany) was applied to the membrane for 1.5 h at 22°C. Finally, the membrane was washed 4×10 min with PBS-TWEEN followed by 3×5 min wash with de-ionized water. Protein bands containing immunoreactive insulin receptor were visualized by exposing the membrane to enhanced chemiluminescence 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). The results are expressed as the mean ± SEM. Statistical comparisons were made using Student's *t*-test.

Figure 1 shows Scatchard plots of insulin binding to epididymal fat tissue plasma membranes of control and MSG rats. Insulin high affinity specific bind-

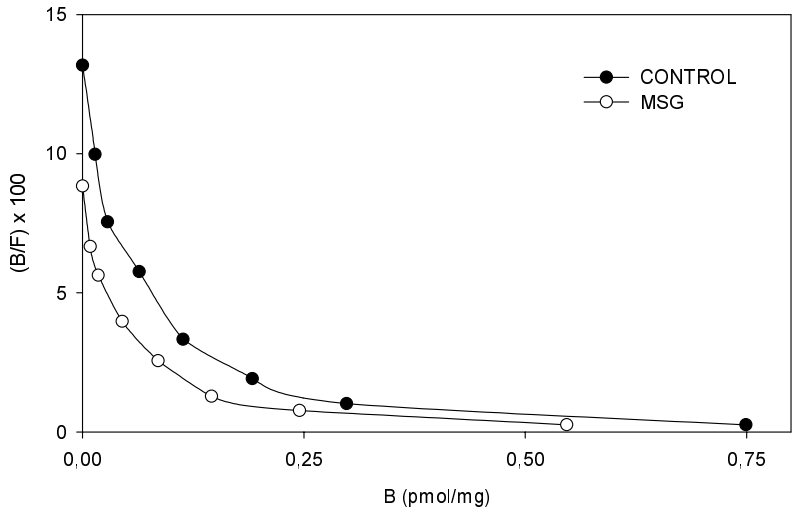

Figure 1. Scatchard plots of ^{125}I-insulin binding to adipose tissue plasma membranes. The values are averages from 10 measurements obtained from two individual experiments. (B/F) $\times 100$, the ratio of bound (B) and free (F) labeled hormone; B, total amount of bound hormone (labeled + nonlabeled) expressed in pmol of insulin *per* mg of membrane protein; MSG, rats with MSG-induced obesity.

ing was significantly lower in obese rats. Analysis of binding curves using two-binding-site model revealed a significantly lower binding capacity (R1: 0.15 ± 0.02 *vs.* 0.26 ± 0.04 pmol/mg, $p < 0.05$, $n = 10$) of high affinity insulin receptors in MSG rats without changes in capacity of low affinity receptors and in affinities of both subtypes of insulin receptor binding sites.

Since adult obese MSG rats display hyperinsulinemia (Pinterova et al. 2001; Macho et al. 2000), the decreased level of high affinity insulin receptors seems to be a typical example of receptor downregulation. The same effect of hyperinsulinemia

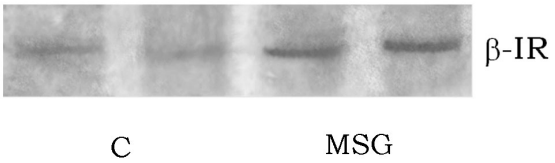

Figure 2. Representative immunoblot of insulin receptor protein in adipose tissue plasma membranes. The same fraction of plasma membranes was used for binding experiment and immunoblot. The immunoblot was performed in two runs with the same pattern of results. Each protein band represents tissue from individual animal. C, control group; MSG, monosodium glutamate induced obesity group; β-IR, beta subunit of insulin receptor.

on high affinity insulin receptors in fat tissue was described in obese spontaneously hypertensive rats (Zorad et al. 2002).

Surprisingly, immunoblot of insulin receptor protein showed higher levels of immunoreactive receptor protein in MSG rats in comparison with controls (19.8 ± 2.4, $n = 4$ *vs.* 5.8 ± 2.1, $n = 3$, $p < 0.01$, expressed in arbitrary units of optical density) (Figure 2).

This discrepancy between lower insulin binding and significantly increased insulin receptor protein can be explained by the existence of impaired insulin receptor protein in MSG-induced obesity. In MSG rats, the same relationship between adipose tissue angiotensin II binding and angiotensin II receptor protein of AT_1 subtype was found (Pinterova et al. 2001). Therefore we assume that in MSG obesity, the general defect of some plasma membrane proteins, e.g. receptors, is present accompanied with extensive translocation of the impaired proteins to plasma membranes. The cause of this defect and the mechanism of extensive translocation of proteins remain to be elucidate.

Acknowledgements. This work was supported by grant of VEGA 2/3190 and in part by grants of VEGA 2/2007 and Centre of Excelence ICA1-CT-2000-70008.

References

Lowry O. H., Rosebrough N. J., Farr A. L., Randall L. (1951): Protein measurement with the Follin phenol reagent. J. Biol. Chem. **193**, 265—275

Macho L., Jezova D., Zorad S., Fickova M. (1999): Postnatal monosodium glutamate treatment results in attenuation of corticosterone metabolic rate in adult rats. Endocr. Regul. **33**, 61—67

Macho L., Fickova M., Jezova D., Zorad S. (2000): Late effect of postnatal administration of monosodium glutamate on insulin action in adult rats. Physiol. Res. (Prague) **49**, (Suppl. 1) S79—85

Munson P. J., Rodbard D. (1980): Ligand: a versatile computerized approach for characterization of ligand-binding systems. In: Computers in Endocrinology (Eds. D. Rodbard and G. Forti), pp. 215—229, Raven Press, New York

Pinterova L., Zelezna B., Fickova M., Macho L., Krizanova O., Jezova D., Zorad S. (2001): Elevated AT_1 receptor protein but lower angiotensin II-binding in adipose tissue of rats with monosodium glutamate-induced obesity. Horm. Metab. Res. **33**, 708—712

Redding T. W., Schally A. V., Arimura A., Wakabayashi I. (1971): Effect of monosodium glutamate on some endocrine functions. Neuroendocrinology **8**, 245—255

Zorad S., Svabova E., Klimes I., Macho L. (1985): Comparison of radiochemical purity and tissue binding of labelled insulin prepared by lactoperoxidase and chloramine T iodination. Endocrinol. Exp. **19**, 267—275

Zorad S., Macho L., Jezova D., Fickova M. (1997): Partial characterization of insulin resistance in adipose tissue of monosodium glutamate-induced obese rats. Ann. N.Y. Acad. Sci. **827**, 541—545

Zorad S., Golda V., Fickova M., Macho L., Pinterova L., Jurcovicova J. (2002): Terguride treatment attenuated prolactin release and enhanced insulin receptor affinity and GLUT4 content in obese spontanously hypertensive female, but not male rats. Ann. N.Y. Acad. Sci. **967**, 1—9

Final version accepted: October 1, 2003