Department of Biochemical Sciences, Charles University, Faculty of Pharmacy in Hradec Králové, Czech Republic

Monosodium glutamate-induced obesity changed the expression and activity of glutathione S-transferases in mouse heart and kidney

I. Boušová*, Š. Košťáková, P. Matoušková, H. Bártíková, B. Szotáková, L. Skálová

Received October 20, 2016, accepted November 30, 2016

**Corresponding author: Iva Boušová, Ph.D., Department of Biochemical Sciences, Charles University, Faculty of Pharmacy, Heyrovského 1203, CZ-500 05 Hradec Králové, Czech Republic*
Iva.Bousova@faf.cuni.cz

Pharmazie 72: 257–259 (2017) *doi: 10.1691/ph.2017.6886*

Obesity may affect activity and/or expression of enzymes participating in xenobiotics' detoxification and antioxidant defense. This study sought to investigate the activities and expression of cardiac and renal glutathione S-transferase (GST) isoforms in order to reveal possible differences between obese and control mice. For this purpose, mice with monosodium glutamate (MSG)-induced obesity were used as an experimental model. Obesity was induced in newborn male mice by repeated s.c. administration of MSG. At 8 months of age, mice were sacrificed and specific activity, protein and mRNA expressions levels of GSTs were analyzed in their heart and kidney. In hearts of obese mice, specific activity of GST was decreased by 51% compared to control. This reduction was accompanied by a decline in GSTP-class protein and Gstp1/2 mRNA expression levels. In contrast, specific activity of GST was elevated by 31% in kidney of obese mice and this increase was accompanied by upregulation of GSTA-class protein and Gsta1/2 mRNA expressions. Increased capacity of renal GSTs together with GSTA upregulation may serve as compensatory mechanism against elevated oxidative stress, which accompanies obesity. On the other hand, decreased cardiac GST activity in obese mice and GSTP downregulation may worsen the defense against oxidative stress and harmful xenobiotics.

1. Introduction

Obesity, a multifactorial disease characterized by excessive fat accumulation, is accompanied by conditions such as type 2 diabetes, dyslipidemia, insulin resistance and non-alcoholic liver steatosis (Ruperez et al. 2014). Various animal models, including monogenic (e.g. *ob/ob* or *db/db* mice) and polygenic models (e.g. diet-induced or chemically induced obesity), are used to study changes in physiological and biochemical parameters in obesity. Polygenic models are more relevant to human obesity as this is mediated by multiple genes (Lutz and Woods 2012). In newborn rodents, repeated subcutaneous administration of monosodium glutamate (MSG) results in obesity development due to hypothalamic *arcuate nucleus* neurons destruction, one of the principal sites regulating energy homeostasis, and produces hyperinsulinemic but hypophagic obesity (Olney 1969). MSG-obese mice have been reported as a suitable model for diabetes, non-alcoholic liver steatosis and metabolic syndrome (Tsuneyama et al. 2014).

Obesity may affect activity and/or expression of enzymes participating in xenobiotic detoxification and antioxidant defense (Matouskova et al. 2015), e.g. glutathione S-transferases (GSTs), a superfamily of crucial xenobiotic-metabolizing enzymes. The most important reaction catalyzed by these enzymes is conjugation of electrophilic xenobiotics with endogenous tripeptide glutathione. Although GST-mediated isomerase and peroxidase reactions have also been reported (Boušová and Skálová 2012). In a previously published work, significant changes in hepatic and intestinal GST activity and expression were observed in MSG-mice (Matouskova et al. 2015).

Our objective, therefore, was to analyze the activities and expressions of cardiac and renal GST isoforms in order to reveal further possible differences between MSG-obese and control mice.

2. Investigations and results

Catalytic activity of GST was assayed using universal substrate 1-chloro-2,4-dinitrobenzene in cardiac cytosolic fractions obtained from control and obese mice. In MSG obese mice, total GST activity was reduced by 51.2% in comparison to control mice. Moreover, this activity decline was accompanied by a significant decrease in GSTP protein level (reduced by 56.9%) and Gstp1/2 mRNA (reduced by 56.0%) level. Results are summarized in Table 1. Protein and mRNA levels of other studied GST isoforms were unaffected.

The activity of GST was also assessed in cytosolic fractions obtained from mouse kidneys. The MSG administration to neonatal mice significantly affected activity as well as expressions of GSTs in renal tissue of obese mice compared to lean controls. In the obese mice, the activity of renal GST was significantly elevated (increase by 31.2%) compared with the non-obese controls. Moreover, the amounts of GSTA protein (7.38-times increased) and Gsta1/2 mRNA (6.44-times elevated) were markedly increased (see Table 1). Protein and mRNA levels of other studied GST isoforms remained unchanged.

3. Discussion

There are indications in the literature that pathologies such as obesity may lead to inter-individual variability in the activity and/or expression of various detoxification enzymes, including GSTs. Such changes may influence the antioxidant balance of organisms as well as the pharmacokinetics and/or pharmacodynamics of therapeutic agents that are administered to obese individuals in order to treat various comorbidities accompanying obesity (Blouin and Warren 1999). In the present study, the effect of experimentally induced obesity on activity and expressions of GSTs was studied in heart and kidney of MSG-obese mice.

In a previous study, the changes in activity and expression of various detoxification enzymes, including GSTs, have been studied in liver and small intestine of MSG-obese mice (Matouskova et al. 2015). In obese mice, hepatic but not intestinal GST activity was diminished compared to controls and this reduction was accompanied by decreased GSTP-class protein and mRNA levels (Matouskova et al. 2015). Similar results were obtained for cardiac tissue in the present study. Ambiguous results on the

Table 1: Catalytic activity, protein and mRNA relative levels of GSTs

	Catalytic activity		Immunoblotting			mRNA quantity		
	control	obese		control	obese		control	obese
Enzyme			**class**			**isoform**		
Heart								
GST	93.6 ± 15.6	45.7 ± 6.1▼	**GSTA**	100.0 ± 3.1	61.5 ± 42.0	**Gsta1/2**[a]	1.00 ± 0.34	3.37 ± 1.71
						Gsta3	1.00 ± 0.40	0.65 ± 0.51
						Gsta4	1.00 ± 0.23	1.08 ± 0.36
			GSTM	100.0 ± 61.0	176.4 ± 42.4	**Gstm3**	1.00 ± 0.53	1.31 ± 0.23
			GSTP	100.0 ± 14.7	43.1 ± 33.9▼	**Gstp1/2**[a]	1.00 ± 0.32	0.44 ± 0.06▼
Kidney								
GST	79.3 ± 10.1	104.0 ± 10.7▲	**GSTA**	100.0 ± 34.1	738.2 ± 278.9▲	**Gsta1/2**[a]	1.00 ± 0.12	6.44 ± 1.25▲
						Gsta3	1.00 ± 0.28	0.94 ± 0.57
						Gsta4	1.00 ± 0.10	1.16 ± 0.19
			GSTM	100.0 ± 26.8	66.8 ± 32.6	**Gstm3**	1.00 ± 0.21	0.69 ± 0.28
			GSTP	100.0 ± 45.3	126.1 ± 51.2	**Gstp1/2**[a]	1.00 ± 0.1 9	1.02 ± 0.31

Abbreviation: GST represents glutathione S-transferase. Specific activity (nmol/min/mg of protein) is expressed as mean ±S.D., where n = 3. Results of immunoblotting and mRNA quantification are expressed as relative mean ± standard deviation (S.D.), controls set to 100%, where n = 4. mRNA quantity expressed as a fold change. Significantly ($P < 0.05$) increased (▲) or decreased (▼) in comparison to control.
[a] Two isoforms analyzed together.

GST activity in obese mice can be found in the literature. While Koide et al. (2011) reported higher total GST activity in liver of obese/diabetic mice, Roe et al. (1999) found lower GST activity in male ob/ob mice compared to non-obese controls. Transcriptomic analysis revealed a significantly lower relative Gstp1/2 expression in heart of obese mice, which corresponds with decreased GSTP-class protein levels, and might be responsible for lower catalytic activity. Human GSTP1 polymorphism was associated with augmented susceptibility to diabetes, abdominal obesity (Amer et al. 2012) and cancer (Helzlsouer et al. 1998). In kidney of MSG-obese mice, total GST activity was remarkably increased and this elevation may be caused by an increase in relative Gsta1/2 expression corresponding to augmented GSTA-class protein level. GSTA1 and GSTA2 isoforms participate in antioxidant defense of organism as they have been reported to possess peroxidase activity towards endogenous products of lipid peroxidation (Boušová and Skálová 2012). Increased capacity of renal GSTs together with GSTA upregulation at protein and mRNA levels may serve as compensatory mechanism against elevated oxidative stress, which accompanies obesity (Ruperez et al. 2014).

In conclusion, repeated administration of MSG to newborn mice caused not only the induction of obesity but also remarkable changes in the activity and expression of GSTs. Effect of MSG on GST activity/expression differed between cardiac and renal tissue. While GST activity was significantly reduced in cardiac tissue, its elevation was found in kidney of obese mice. In heart of obese mice, protein and mRNA levels of GSTP class enzymes were decreased. In contrast, GST activity elevation observed in kidney was accompanied with increased protein and mRNA levels of GSTA class enzymes. Such changes in the activity/expression of GSTs could seriously influence detoxification of xenobiotics, metabolism of numerous endogenous compounds and antioxidant defense of organism.

4. Experimental

4.1. Experimental animals

Male NMRI mice obtained from Meditox (Konárovice, Czech Republic) were housed in air-conditioned animal quarters, with a 12 h light/dark cycle (light from 6 AM). They were given *ad libitum* access to water and standard chow diet. The mice were cared for and used in accordance with the Guide for the Care and Use of Laboratory Animals (Protection of Animals from Misuse Act No. 246/92, Czech Republic). The Ethical Committee of Charles University in Prague, Faculty of Pharmacy in Hradec Králové, approved all animal experiment procedures (permit number: 34354/2010-30).

Newborn male mice were divided into two groups (n = 12): control and obese mice. Details of study design have been published (Matouskova et al. 2015). At 8 months of age, all mice were fasted for 12 h, anesthetized and killed by cervical dislocation. After thoracotomy, kidney and heart were removed, washed with PBS containing proteases inhibitors cocktail (Roche, Mannheim, Germany) and immediately frozen in liquid nitrogen. Small pieces from each tissue were separately placed in RNAlater solution (Qiagen, Austin, TX, USA). All biological samples were stored in a freezer at -80 °C.

4.2. Procedures

4.2.1. Preparation of subcellular fractions

Cytosolic fractions were obtained from the nitrogen-frozen hearts and kidneys of the mice by fractional ultracentrifugation as described earlier (Matouskova et al. 2015). All subcellular fractions were stored at −80 °C. The protein concentrations in the cytosolic fractions were assayed using the bicinchoninic acid method, according to the manufacturer's instructions (Sigma-Aldrich, Prague, Czech Republic).

4.2.2. Activity assessment

Total cytosolic GST activity was assayed by spectrophotometric method adapted for 96-well plate using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced glutathione as substrates according to published methods (Habig and Jakoby 1981; Boušová et al. 2012). Enzyme assays were performed in three independent experiments, with 8 parallel measurements for each sample.

4.2.3. Protein expression

Cytosolic GSTA, GSTM, and GSTP isoforms protein expression levels in mice hearts and kidneys were determined using Western blot analysis according to previously published methods (Towbin et al. 1979; Matouskova et al. 2015). Primary antibodies (goat polyclonal to GSTA at dilution 1:3000; goat polyclonal to GSTM at dilution 1:3000; rabbit monoclonal to GSTP at dilution 1:5000) and secondary antibodies (bovine to rabbit IgG and bovine to goat IgG, both diluted at 1:10,000) were obtained from Abcam (Cambridge, UK) and Santa Cruz Biotechnology (Santa Cruz, TX, USA), respectively. γ-Tubulin (rabbit monoclonal at dilution 1:2000) served as the loading control. Band intensities were evaluated using an Image studio Lite Ver 3.1.

4.2.4. RNA extraction, cDNA synthesis and quantitative Real-Time PCR

Approximately 50 mg of heart or kidney tissue was used for total RNA extraction using TriReagent, according to the manufacturer´s instructions (Molecular Research Center, Cincinnati, OH, USA). Upon RNA concentration and purity determination, the first strand cDNA synthesis was performed using ProtoScript II reverse transcriptase (NEB, Whitby, ON, Canada) and random hexamers as described previously (Matouskova et al. 2015). Real-time quantitative PCR (qPCR) analyses were performed in an iQ5 thermocycler (Bio-Rad) using a qPCR Core kit with SYBR Green I detection (Invitrogen, Carlsbad, CA, USA) as described in previous study (Matouskova et al. 2015). The primers for individual GSTs were designed manually and are listed in Table 2.

4.3. Statistical analyses

The assays of enzymatic activity and protein expression were determined using a cytosolic fraction of pooled samples (n = 3-4). Gene expression was analyzed using data from at least four biological replicates in each group. Relative and normalized fold expression values were calculated manually in Microsoft Excel from the Cq values imported from qPCR software. All mRNA levels were expressed as percentage changes relative to the controls using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The relative mRNA levels were normalized to quantification cycle values of glyceraldehyde 3-phosphate dehydrogenase reference gene.

All data were imported to GraphPad Prism (GraphPad Software, version 6.0) and the statistical significance of the results was analyzed with Student's t-test with Welch's correction. Differences were scored as statistically significant at the $P < 0.05$.

Acknowledgements: This work was supported by the Czech Science Foundation [Centre of Excellence, grant number P303/12/G163] and by the Charles University

Table 2 Target and reference genes selected for qPCR. National Center for Biotechnology Information (NCBI) reference sequences, primers and amplicon sizes.

Gene	NCBI Accession No.	Forward primer	Reverse primer	Amplicon size
Gsta1/2	NM_008181 / NM_008182	GATTGGGCAATTGGTATTATGTC	CCTGTTGCCCACAAGGTAGT	142
Gsta3	NM_010356	GACCTGGCAAGGTTACGAAG	TATCTCCAGATCCGCCACTC	195
Gsta4	NM_010357	CCTCGCTGCCAAGTACAACT	TTGCCAACGAGAAAAGCCTC	231
Gstm3	NM_010359	GCTCATGATAGTCTGCTGCAG	GCTTCATTTTCTCAGGGATGGC	80
Gstp1/2	NM_013541 / NM_181796	AGCCTTTTGAGACCCTGCTG	CGGCAAAGGAGATCTGGTCA	75

[Research project SVV 260 294]. The authors would like to thank Dr. V. Hanušová for helping in handling the animals and collecting tissues.

Conflicts of interest: None declared.

References

Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH (2012) Evaluation of glutathione S-transferase P1 genetic variants affecting type-2 diabetes susceptibility and glycemic control. Arch Med Sci 8: 631-636.

Blouin RA, Warren GW (1999) Pharmacokinetic considerations in obesity. J Pharm Sci 88: 1-7.

Boušová I, Hájek J, Dršata J, Skálová L (2012) Naturally occurring flavonoids as inhibitors of purified cytosolic glutathione S-transferase. Xenobiotica 42: 872-879.

Boušová I, Skálová L (2012) Inhibition and induction of glutathione S-transferases by flavonoids: possible pharmacological and toxicological consequences. Drug Metab Rev 44: 267-286.

Habig WH, Jakoby WB (1981) Glutathione S-transferases (rat and human). Methods Enzymol 77: 218-231.

Helzlsouer KJ, Selmin O, Huang HY, Strickland PT, Hoffman S, Alberg AJ, Watson M, Comstock GW, Bell D (1998) Association between glutathione S-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. J Natl Cancer Inst 90: 512-518.

Koide CL, Collier AC, Berry MJ, Panee J (2011) The effect of bamboo extract on hepatic biotransforming enzymes-findings from an obese-diabetic mouse model. J Ethnopharmacol 133: 37-45.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

Lutz TA, Woods SC (2012) Overview of animal models of obesity. Curr Protoc Pharmacol 58: 5.6.1-18.

Matouskova P, Bartikova H, Bousova I, Levorova L, Szotakova B, Skalova L (2015) Drug-metabolizing and antioxidant enzymes in monosodium L-glutamate obese mice. Drug Metab Dispos 43: 258-265.

Olney JW (1969) Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164: 719-721.

Roe AL, Howard G, Blouin R, Snawder JE (1999) Characterization of cytochrome P450 and glutathione S-transferase activity and expression in male and female ob/ob mice. Int J Obes Relat Metab Disord 23: 48-53,

Ruperez AI, Gil A, Aguilera CM (2014) Genetics of oxidative stress in obesity. Int J Mol Sci 15: 3118-3144.

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets - procedure and some applications. Proc Natl Acad Sci U S A 76: 4350-4354.

Tsuneyama K, Nishida T, Baba H, Taira S, Fujimoto M, Nomoto K, Hayashi S, Miwa S, Nakajima T, Sutoh M, Oda E, Hokao R, Imura J (2014) Neonatal monosodium glutamate treatment causes obesity, diabetes, and macrovesicular steatohepatitis with liver nodules in DIAR mice. J Gastroenterol Hepatol 29: 1736-1743.