

Sterol Regulatory Element Binding Protein 2 Overexpression Is Associated With Reduced Adipogenesis and Ectopic Fat Accumulation in Transgenic Spontaneously Hypertensive Rats

V. LANDA¹, V. ZÍDEK¹, P. MLEJNEK¹, M. ŠIMÁKOVÁ¹, J. ŠILHAVÝ¹, J. TRNOVSKÁ^{2,3}, L. KAZDOVÁ², M. PRAVENEČ¹

¹Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic,

²Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic, ³Czech University of Life Sciences, Faculty of Agrobiolgy, Food and Natural Resources, Department of Veterinary Sciences, Prague, Czech Republic

Received February 12, 2014

Accepted March 21, 2014

On-line June 5, 2014

Summary

It has been reported that the major function of the sterol regulatory element binding protein 2 (SREBP-2) is to activate preferentially cholesterol biosynthesis in liver and adipose tissue rather than fatty acid synthesis. In the current study, we analyzed the effects of overexpression of human dominant-positive SREBP-2 transgene under control of PEPCK promoter in the spontaneously hypertensive rat (SHR) on lipid and glucose metabolism. Transgenic overexpression of SREBP-2 was associated with significantly higher hepatic triglycerides (20.4 ± 0.9 vs. 17.0 ± 0.05 $\mu\text{mol/g}$, $P < 0.05$) but not cholesterol (10.6 ± 0.4 vs. 10.9 ± 0.4 $\mu\text{mol/g}$) and decreased relative weight of epididymal fat pad (0.73 ± 0.03 vs. 0.83 ± 0.03 , $P < 0.05$). In addition, muscle triglyceride (15.8 ± 3.7 vs. 8.5 ± 1.2 $\mu\text{mol/g}$, $P < 0.001$) and cholesterol (3.6 ± 0.5 vs. 2.1 ± 0.1 $\mu\text{mol/g}$, $P < 0.05$) concentrations were significantly increased in transgenic rats when compared to SHR controls. Ectopic fat accumulation was associated with significantly increased serum glucose levels (6.4 ± 0.1 vs. 5.9 ± 0.1 mmol/l , $P < 0.005$) and reduced insulin levels (1.78 ± 0.33 vs. 2.73 ± 0.37 nmol/l , $P < 0.05$) in transgenic rats. These results provide evidence for important role of SREBP-2 in regulation of lipid and glucose metabolism.

Key words

Sterol regulatory element binding protein 2 • Transgenic • Spontaneously hypertensive rat • Triglycerides • Cholesterol

Corresponding author

M. Pravenec, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: pravenec@biomed.cas.cz

Introduction

The spontaneously hypertensive rat (SHR) is the most widely used animal model of essential hypertension and under special environmental condition, such as when fed a high sucrose diet, also develops disturbances of lipid and glucose metabolism (Pravenec *et al.* 2014). Sterol regulatory element binding proteins (SREBPs) belong to a family of transcription factors that play important role in regulation of cholesterol and fatty acid biosynthesis. There are 3 SREBPs that are coded by 2 genes. *Srebf1* (sterol regulatory binding protein factor 1) codes for 2 isoforms, SREBP-1a and SREBP-1c, by using alternate promoters. The second gene, *Srebf2* (sterol regulatory element binding protein 2) codes for SREBP-2 transcription factor (Brown and Goldstein 1997). While all 3 SREBPs activate similar sets of genes they differ in their effects on fatty acid and cholesterol metabolism. The role of these 3 isoforms in regulation of lipid metabolism was tested in mice and rats that expressed dominant-positive SREBP transgenes under control of the PEPCK promoter. Both mice and rats expressing SREBP-1a transgenic isoform exhibited

extreme hepatic steatosis due to increased triglyceride and cholesterol content and reduced adiposity (Shimano *et al.* 1996, Qi *et al.* 2005, Malinská *et al.* 2010) which suggested that SREBP-1a isoform plays an important role in regulation of both cholesterol and fatty acid synthesis in the liver. On the other hand, mice and rats expressing a dominant-positive SREBP-1c isoform showed only modest increase in hepatic triglycerides and cholesterol (Shimano *et al.* 1997, Pravenec *et al.* 2008). Finally, studies with mice expressing dominant-positive SREBP-2 isoform provided evidence that the major role of SREBP-2 is to activate cholesterol biosynthesis in preference to fatty acid synthesis in liver and adipose tissue (Horton *et al.* 1998). In the current study, we analyzed metabolic effects of overexpressing dominant-positive SREBP-2 isoform in the spontaneously hypertensive rat (SHR). We found that expression of the dominant-positive SREBP-2 is associated with increased hepatic triglycerides but not cholesterol, reduced adipogenesis, and with ectopic fat accumulation in muscles in the SHR.

Methods

Generation of transgenic rats

Transgenic rats overexpressing dominant-positive form of human SREBP-2 were derived on genetic background of the SHR/Ola strain. Transgenic SHR were produced by microinjection of ova with a cDNA construct encoding amino acids 1 to 468 of human SREBP-2 under control of the rat PEPCK (phosphoenolpyruvate carboxykinase) promoter, which produces high levels of gene expression in the liver (SREBP-2 construct was kindly provided by J. Horton and J. Goldstein). The cDNA transgene generates a truncated form of SREBP-2 that can enter the nucleus without proteolysis and is not subject to feedback regulation (Horton *et al.* 1998). The rats were housed in an air-conditioned animal facility and allowed free access to food and water. We used males (N=8 per group) at the age of 4 months. Rats were fed a standard chow till 14 weeks of age and 2 weeks prior to experiment a diet containing 60 % fructose. A high fructose diet was used in these studies based on previous work indicating that such diets facilitate the development of metabolic disturbances in SHR models (Pravenec *et al.* 2014). All protocols were performed in agreement with the Animal Protection Law of the Czech Republic and were approved by the Ethics Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

Expression analysis of transgenic and endogenous SREBP-2 genes

Real-time PCR analysis was used to detect expression of the human SREBP-2 transgene. For real-time PCR, total RNA was isolated using standard methods and cDNA was prepared and analyzed by real-time PCR testing using QuantiTect SYBR Green reagents (Qiagen, Inc, Valencia, U.S.A.) on an Opticon continuous fluorescence detector (MJ Research, Waltham, U.S.A.). Gene expression levels were normalized relative to the expression of cyclophilin (peptidylprolyl isomerase A), which served as the internal control, with results being determined in triplicate. Expression of the transgene encoding human SREBP-2 was quantified using the following primers: upstream, 5'-GAG ACC ATG GAG ACC CTC AC; downstream, 5'-CAG GGA ACT CTC CCA CTT GA. Expression of endogenous gene encoding rat SREBP-2 was quantified using the following primers: upstream, 5'-ATC ATC CAG CAG CCT TTG AT; downstream, 5'-CTG TAC CTG CGA GGA TGT CA.

Tissue triglyceride measurements

For determination of triglycerides in liver and soleus muscle, tissues were powdered under liquid N₂ and extracted for 16 h in chloroform:methanol, after which 2 % KH₂PO₄ was added and the solution was centrifuged. The organic phase was removed and evaporated under N₂. The resulting pellet was dissolved in isopropyl alcohol, and triglyceride content was determined by enzymatic assay (Erba-Lachema, Brno, Czech Republic).

Biochemical analyses

Biochemical parameters were measured in blood samples drawn from non-fasting rats. Blood glucose levels were measured by the glucose oxidase assay (Erba-Lachema, Brno, Czech Republic) using tail vein blood drawn into 5 % trichloroacetic acid and promptly centrifuged. NEFA levels were determined using an acyl-CoA oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum triglyceride and cholesterol concentrations were measured by standard enzymatic methods (Erba-Lachema, Brno, Czech Republic). Serum insulin concentrations were determined using the Mercodia Rat Insulin ELISA kit (Mercodia AB, Uppsala, Sweden).

Oral glucose tolerance testing

Oral glucose tolerance tests were performed using a glucose load of 300 mg/100 g body weight after

overnight fasting. Blood was drawn from the tail without anesthesia before the glucose load (0 min time point) and at 30, 60, and 120 min thereafter.

Statistical analysis

We performed the statistical analysis using *t*-tests or rank sum tests for two group comparisons. All results are expressed as means \pm standard errors of the means (SEM).

Results

Expression of the human SREBP-2 transgene and *Srebf2* endogenous gene

Real-time PCR analysis confirmed abundant hepatic expression of the human transgene encoding SREBP-2 compared with expression of the endogenous *Srebf2* gene (Fig. 1). Lower expression of the transgene and *Srebf2* endogenous gene was detected also in adipose tissue.

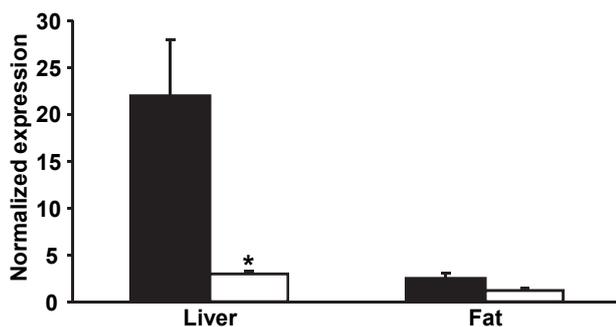


Fig. 1. Gene expression levels. Real-time PCR analysis of SHR transgenic rats showing tissue expression levels of the transgene for SREBP-2 (solid bars) compared with expression levels of the endogenous gene for SREBP-2 (open bars) in liver and adipose tissue. * denotes $P < 0.05$.

Metabolic effects of transgenic expression of SREBP-2

As can be seen in Table 1, transgenic rats when compared to nontransgenic controls, had significantly higher body weight but lower weight of relative epididymal fat pad. Serum triglycerides were increased in transgenic rats while no significant differences were found in serum NEFA and cholesterol. Transgenic rats had higher hepatic triglyceride but not cholesterol concentrations versus SHR controls. On the other hand, muscle triglycerides and cholesterol were increased in transgenic rats which was associated with significantly higher glucose and reduced insulin levels. In addition, transgenic rats had higher levels of NEFA during OGTT

which suggests reduced antilipolytic action of insulin (Table 1). No significant differences were observed in serum glucose or insulin concentrations during OGTT (data not shown).

Table 1. Metabolic phenotypes in SHR-*Srebf2* transgenic versus SHR controls.

Trait	SHR- <i>Srebf2</i>	SHR
Body weight (g)	327 \pm 7	304 \pm 6*
Rel. epididymal fat weight (g/100 g BW)	0.73 \pm 0.03	0.83 \pm 0.03*
Serum triglycerides (mmol/l)	2.10 \pm 0.10	1.40 \pm 0.08*
Serum NEFA (mmol/l)	0.57 \pm 0.06	0.64 \pm 0.09
Serum NEFA during OGTT (mmol/l)	0.81 \pm 0.03	0.59 \pm 0.04*
Serum glucose (mmol/l)	6.4 \pm 0.1	5.9 \pm 0.1**
Serum insulin (nmol/l)	1.8 \pm 0.3	2.7 \pm 0.4*
Serum cholesterol (mmol/l)	1.6 \pm 0.1	1.7 \pm 0.1
Liver triglycerides (μ mol/g)	20.4 \pm 0.9	17.0 \pm 0.5*
Liver cholesterol (μ mol/g)	10.6 \pm 0.4	10.9 \pm 0.4
Muscle triglycerides (μ mol/g)	14.1 \pm 1.5	9.4 \pm 1.4*
Muscle cholesterol (μ mol/g)	3.6 \pm 0.5	2.1 \pm 0.1*

* and ** denote $P < 0.05$ and $P < 0.005$, respectively.

Discussion

In our previous studies with SREBP-1a and SREBP-1c dominant-positive transgenes expressed on genetic background of the spontaneously hypertensive rat (SHR), we observed significant effects on lipid and glucose metabolism. Overexpression of SREBP-1a isoform was associated with striking accumulation of triglycerides in the liver, oxidative stress, and steatohepatitis (Qi *et al.* 2005, Malinská *et al.* 2010). On the other hand, overexpression of the SREBP-1c isoform in the SHR, which harbors a mutant form of the *Srebf1* gene that predisposes the SHR to hepatic steatosis, was

used for a transgenic rescue experiment (Pravenec *et al.* 2008). In the current study, we analyzed metabolic effects associated with overexpression of the dominant-positive SREBP-2 transgene. SREBP-2 transgenic rats when compared to nontransgenic controls exhibited significantly increased hepatic levels of triglycerides but not cholesterol, lower adiposity, and increased triglyceride and cholesterol content in muscles. Ectopic fat accumulation in livers and muscles was associated with increased levels of nonfasting glucose and reduced insulin. Although we did not measure lipid content in pancreatic β -cell cell it is possible that ectopic fat accumulation in pancreas leads to dysregulated insulin secretion (Kazdová *et al.* 2002).

Contrary to mice that overexpressed the same dominant-positive SREBP-2 transgene (Horton *et al.* 1998), we observed no increase in hepatic cholesterol levels which does not support the major role of SREBP-2 in cholesterol synthesis in the liver. In addition, we

observed reduced adiposity in transgenic rats associated with ectopic fat accumulation and insulin resistance. Such discrepant results might be explained by different genetic backgrounds (rats versus mice) and different diets (high fructose versus high protein/low carbohydrate). It can be concluded that overexpression of SREBP-2 in the SHR fed a high fructose diet is associated with reduced white adipose tissue mass and ectopic fat accumulation in livers and muscles but not with significant differences in cholesterol content in tissues.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by grant LH12061 from Ministry of Education, Youth, and Sports of the Czech Republic to V.L.

References

- BROWN MS, GOLDSTEIN JL: The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**: 331-340, 1997.
- HORTON JD, SHIMOMURA I, BROWN MS, HAMMER RE, GOLDSTEIN JL, SHIMANO H: Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J Clin Invest* **101**: 2331-2339, 1998.
- KAZDOVÁ L, DIVIŠOVÁ J, CAHOVÁ M, MARKOVÁ I: Aging-induced impairment of insulin secretion is associated with increased islet triglyceride content in non-obese hereditary hypertriglyceridemic rats. *Diabetologia* **45** (Suppl 2): 228, 2002.
- MALÍNSKÁ H, OLIYARNYK O, HUBOVÁ M, ZÍDEK V, LANDA V, ŠIMÁKOVÁ M, MLEJNEK P, KAZDOVÁ L, KURTZ TW, PRAVENEK M: Increased liver oxidative stress and altered PUFA metabolism precede development of non-alcoholic steatohepatitis in SREBP-1a transgenic spontaneously hypertensive rats with genetic predisposition to hepatic steatosis. *Mol Cell Biochem* **335**: 119-125, 2010.
- PRAVENEK M, KAZDOVÁ L, LANDA V, ZÍDEK V, MLEJNEK P, ŠIMÁKOVÁ M, JANSÁ P, FOREJT J, KŘEN V, KŘENOVÁ D, QI N, WANG JM, CHAN D, AITMAN TJ, KURTZ TW: Identification of mutated Srebf1 as a QTL influencing risk for hepatic steatosis in the spontaneously hypertensive rat. *Hypertension* **51**: 148-153, 2008.
- PRAVENEK M, KŘEN V, LANDA V, MLEJNEK P, MUSILOVÁ A, ŠILHAVÝ J, ŠIMÁKOVÁ M, ZÍDEK V: Recent progress in the genetics of spontaneously hypertensive rats. *Physiol Res* **63** (Suppl 1): S1-S8, 2014.
- QI NR, WANG J, ZÍDEK V, LANDA V, MLEJNEK P, KAZDOVÁ L, PRAVENEK M, KURTZ TW: A new transgenic rat model of hepatic steatosis and the metabolic syndrome. *Hypertension* **45**: 1004-1011, 2005.
- SHIMANO H, HORTON JD, HAMMER RE, SHIMOMURA I, BROWN MS, GOLDSTEIN JL: Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* **98**: 1575-1584, 1996.
- SHIMANO H, HORTON JD, SHIMOMURA I, HAMMER RE, BROWN MS, GOLDSTEIN JL: Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* **99**: 846-854, 1997.