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Short communication

Hepatic and Pancreatic Glycosphingolipid Phenotypes of the Neurological Different Rat Strains

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A B S T R A C T

Among three commonly used strains of laboratory rats, Wistar rats perform more neurological tasks better then Lewis and Sprague-Dawley (SD) rats. Liver is the main site of insulin-like growth factor (IGF) production and pancreas is the exclusive site of insulin production. Insulin stimulates neuronal development and appropriate IGF-I input is critical in brain growth. Glycosphingolipids (GSLs) are important mediators of insulin secretion and action. Therefore, this study investigated GSL phenotypes of liver and pancreas with hypothesis that they are different in three rat strains. Total GSL fractions (neutral and gangliosides) were analysed by high performance thin-layer chromatography (HPTLC). Complex gangliosides were detected by HPTLC immunostaining using cholera toxin B subunit after neuraminidase pretreatment. Wistar rats had the highest liver weight/body weight ratio and SD rats had the highest pancreas weight/body weight ratio. Ganglioside GM3 was more expressed in the liver of Wistar compared to Lewis and SD rats. SD rats contained scarce quantities of GD1a and b-series gangliosides in the liver compared to Wistar and Lewis rats. Pancreatic b-series ganglioside content was also the lowest in SD rats. This study represents differences in the hepatic and pancreatic ganglioside phenotypes of three rat strains that could influence IGF and insulin secretion and action.

Key words: *b-series gangliosides, GM3,GD1a, liver, pancreas, rat strains*

Introduction

Morphologic, sensory and motor differences exist between the Wistar, Lewis, and Sprague-Dawley (SD) rats[1]. Wistar rats have enhanced biosynthesis of triacylglycerol from naturally occurred cis fatty acids compared to SD rats[2]. When rats fast for 2 days, ketogenesis from cis fatty acids is slower in Wistar compared to SD strain[2]. The stimulation index of Lewis pancreatic islets after glucose challenge is almost three times as high as that of Wistar islets[3]. Liver is the main site of insulin-like growth factor (IGF) synthesis although it could be produced locally by most cell types[4]. IGF play a particular role in the trophic maintenance of neurons involved in the coordination of sensorimotor function in the cerebellum[5]. Pancreas is the exclusive site of insulin production, and the role of insulin during development, although not well defined yet, may be related to the control of neuronal survival[6]. Sialic acid-containing glycosphingolipids, gangliosides, are known to modulate the activity of a number of receptor tyrosine kinases, including the insulin receptor[7]. Inhibition of glycosphingolipid synthesis can significantly improve insulin sensitivity and glucose homeostasis[8]. The tyrosine kinase activity of the epidermal growth factor receptor can be enhanced or repressed by gangliosides GD1a or GM3, respectively[9,10]. Therefore, our hypothesis was that Wistar, Lewis, and SD rats differed in their hepatic and pancreatic glycosphingolipid phenotypes. In this study, we examined expression of two GSL subclasses: ceramide monohexosides and gangliosides in the liver and pancreas of one month old Wistar, Lewis and SD rats.

Materials and Methods

Animals

Experiments were performed on one month old male Wistar (weighting 94.2±8.9 g, mean±standard deviation), Lewis (85.4±14.3) and Sprague-Dawley rats (71.9±8.5)

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(n=10, for each rat strain). All rats were raised under controlled conditions (temperature, 22±1°C; light schedule: 14h of light and 10h of dark) at University of Split Animal Facility. Laboratory food and tap water were supplied *ad libitum*. Animals were bred and maintained according to the NIH Guide for the Use and Care of Laboratory Animals and the University Medical School Ethics Committee has approved the experimental protocol. For GSL analysis, rats were sacrificed with prolonged anesthesia (diethyl ether); the tissues were dissected out and stored at –20°C until GSL extraction.

Isolation of GSLs from tissues

Liver and pancreas were dissected from 10 animals of each group. Identical tissues were pooled, minced with a scalpel, suspended in distilled water in a 1/2 ratio (w/v), homogenized and isolated according to standard procedures[11]. Briefly, GSLs were extracted with chloroform/methanol (2/1, v/v), chloroform/methanol (1/1, v/v) and chloroform/methanol (1/2, v/v) (10-fold volumes of the tissue wet weight) for 30 min with sonication. The combined extracts were dried and phospholipids were saponified with aqueous 1 M NaOH for 1 h at 37°C. After neutralization with 1 M HCl, the samples were dialyzed against deionized water and dried[12]. The extracts were adjusted to defined volumes of chloroform/methanol (2/1, v/v) corresponding to 2 mg wet tissue weight/mL for all tissues.

High performance thin layer chromatography

The gangliosides were separated on silica gel 60 pre-coated high performance thin layer chromatography plates (HPLTC-plates, size 10x10cm, and thickness 0.2 mm, Merck, Darmstadt, Germany) in the solvent chloroform/methanol/water (120/70/17, each by vol., with 2 mM $CaCl_2$) and visualized with orcinol[12]. Orcinol staining was performed three times, with identical results.

Detection of GM1a-type gangliosides by HPTLC immunostaining

The HPTLC binding assay using cholera toxin B subunit (= choleragenoid) for specific detection of GM1 was developed by Magnani et al.[13] and was used with modifications[14,15]. A fixed silica gel plate with polyisobutylmethacrylate was overlayed with 250 ng/mL choleragenoid (Sigma, Deisenhofen, Germany), diluted in solution A (phosphate-buffered saline, PBS, supplemented with 1% bovine serum albumin and 0.02% NaN_3) for 2 h at room temperature. Unbound choleragenoid was removed by washing of plates five times with solution B (0.05% Tween 21, 0.02% NaN_3 in PBS) followed by rabbit anti-choleragenoid and secondary goat anti-rabbit IgG antibody incubation, both diluted 1:2000 (both from Abcam Cambrige, UK). After 1 h of incubation with secondary antibody, the plates were washed again, followed by two rinses with glycine buffer (0.1M glycine, 1mM $ZnCl_2$, 1mM $MgCl_2$, pH 10.4), to remove phosphate. Bound choleragenoid was visualized with 0.05% (w/v) 5-bromo-4-chloro-3-indolylphosphate (Biomol, Hamburg, Germany) in glycine buffer. To reveal the presence of GD1a, GD1b, GT1b, and GQ1b the plates were incubated with 5 mU/mL *V. cholerae* neuraminidase (Sigma, Deisenhofen, Germany) (2 h, 37°C) prior to combined choleragenoid immunostaining[14]. Choleragenoid immunostaining with neuraminidase pretreatment was performed three times, with identical results.

Estimation of relative quantities of ganglioside fractions was performed by AutoCAD software (Autodesk, GmbH, Germany).

Statistical analysis

Data of body weight and the organ weight/ body weight ratio are given as means±standard deviations. Repeated measures ANOVA with Tukey-Kramer post-hoc test were performed using GraphPad Prism version 4.00 for Windows (San Diego, USA).

These tests were chosen due to the small number (n=10) of the samples in the each group.

Results

Liver weight/body weight ratio was significantly higher in Wistar compared to other two strains ($p<0.05$, Table 1). There was no difference between Lewis and SD liver weight/body weight ratio. Pancreas weight/body weight ratio was significantly higher in SD compared to other two strains ($p<0.05$). There was no difference between Wistar and Lewis pancreas weight/body weight ratio. Staining of HPTL-chromatograms with orcinol revealed that major neutral glycosphingolipids in the liver of all strains were two ceramide monohexoside fractions and one GSL band chromatographed at the position of Gb4Cer (Figure 1). Ganglioside GM3 was present only in traces, not detectable by densitometric software, in Lewis

TABLE 1
BODY WEIGHT AND ORGAN WEIGHT/BODY WEIGHT RATIOS IN WISTAR (W), LEWIS (L) AND SPRAGUE-DAWLEY (SD) RATS

	Wistar	Lewis	Sprague-Dawley	ANOVA	
				F	p
Liver weight/body weight (g/g)	*,**0.0521±0.0029	**0.0407±0.0025	*0.0412±0.0044	36.32	<0.0001
Pancreas weight/body weight (g/g)	***0.0033±0.0004	****0.0035±0.0005	***,****0.0042±0.0006	6.484	<0.05

Data are reported as means±standard deviations for 10 samples in each group. Tukey's Multiple Comparison test
$^{*}p<0.05$ W vs SD; $^{**}p<0.05$ W vs L; $^{***}p<0.05$ W vs SD; $^{****}p<0.05$ L vs SD

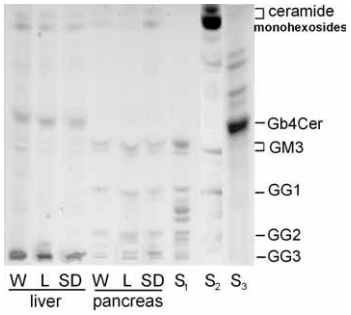

Fig. 1. Orcinol stain of HPTLC-separated GSL fractions from liver and pancreas of Wistar (W), Lewis (L) and Sprague-Dawley (SD) rats. GSL amounts corresponding to 30 mg liver and pancreas wet weight were chromatographed together with reference GSLs, S1 and S2. S1: 5 μg of gangliosides from rat brain, S2: 10 μg of neutral GSLs from mouse brain, and S3: 5 μg of neutral GSLs from human erythrocytes.

and SD liver, while proportion of GM3 in total GSL content was 14% in the Wistar liver (1051/sum of all GSL fractions, Figure 2). Low level of GM3 detection in Lewis and SD liver was due to low quantity of hepatic GSL extracts applied (corresponding to 30 mg liver wet weight).

Choleragenoid stain of hepatic and pancreatic ganliosides is shown at Figure 3. Cholera toxin B subunit preferentially reacts with GM1 and after neuraminidase treatment also with gangliosides derived from gangliotetraose. As the reaction does not have to run quantitatively, colour response of individual gangliosides can be rather different. It is possible to compare quantitative changes of each individual ganglioside but not proportions among them. Lower GD1a ganglioside fraction was 6 fold and 4 fold higher expressed in Wistar and Lewis liver, respectively, compared to SD liver (Figure 3 and Figure 4). The results on densitometric analysis of ganglioside detected with choleragenoid are given in graphical form only for the fractions that were different more then 2 fold between the strains (Figure 4 and Figure 5). The separation of individual rat gangliosides on HPTLC plates as double bands is common feature, due to the variation in the ceramide portion (C16- or C-24 fatty acids). The upper band is composed mainly of C-24 fatty acid, and the lower band of C-16 fatty acid[15]. Ganglioside GD1b was 4 fold higher expressed in Wistar liver compared to SD liver (Figures 3 and 4). The difference in b-series ganglioside expression was more obvious in pancreas. Wistar pancreas showed 5 fold higher amount of both GD1b and GT1b ganglioside compared to SD pancreas. Lewis strain contained higher pancreatic GD1b, being 6 fold increased compared to SD rats (Figures 3 and 5).

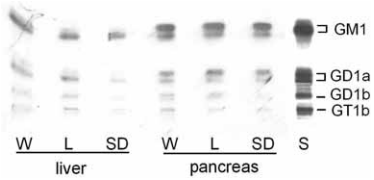

Fig. 3. Immunostain with choleragenoid after V. cholerae neuraminidase treatment of HPTLC-separated ganglioside fractions from liver and pancreas of Wistar (W), Lewis (L) and Sprague-Dawley (SD) rats. Ganglioside amounts corresponding to 5 mg liver and pancreas wet weight were chromatographed together with 167 ng of reference gangliosides from rat brain.

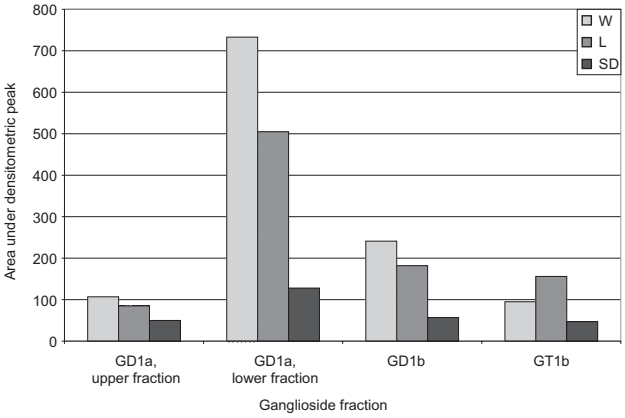

Fig. 4. Areas under densitometric peaks of distinct hepatic ganglioside fractions in Wistar (W), Lewis (L) and Sprague-Dawley (SD) rats determined with choleragenoid.

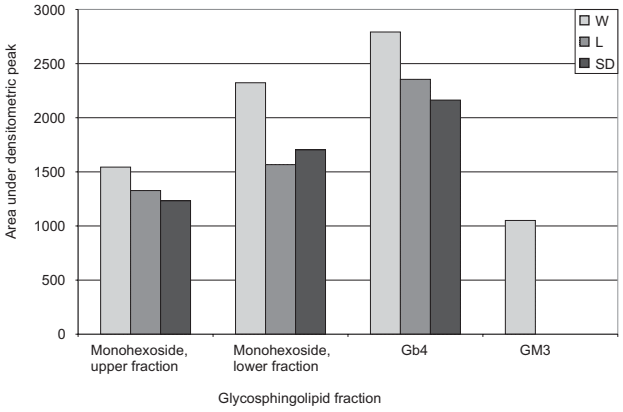

Fig. 2. Areas under densitometric peaks of distinct hepatic ganglioside fractions in Wistar (W), Lewis (L) and Sprague-Dawley (SD) rats determined with orcinol staining.

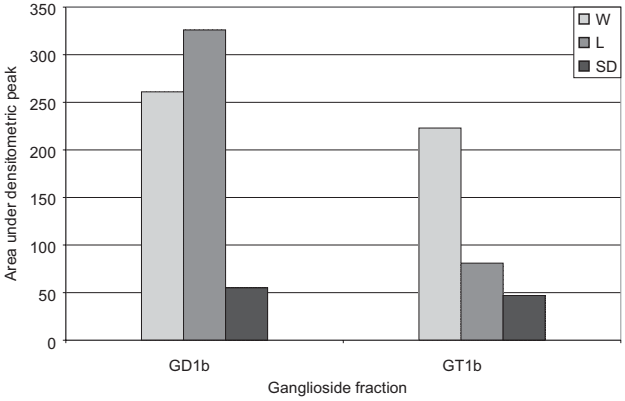

Fig. 5. Areas under densitometric peaks of distinct pancreatic ganglioside fractions in Wistar (W), Lewis (L) and Sprague-Dawley (SD) rats determined with choleragenoid.

Discussion

This study showed some differences in hepatic and pancreatic ganglioside phenotypes in Wistar, Lewis, and SD rats. Rat hepatic gangliosides are well described by Kasai et al.[16] and pancreatic gangliosides by Dotta et al.[17]. The pattern of ganglioside distribution in Lewis rat liver is already classified as a-type, which is characterized by dominance of a-series gangliosides[16]. In Wistar Furth pancreas, the main ganglioside fractions are GM3, GD3, GD1a, GT1b and a trisialoganglioside migrating above the GT1b standard (42.7, 7, 20.2, 13.8, and 6.8, respectively)[17]. Since GM3 and GD1a gangliosides are known to modulate the activity of epidermal growth factor receptor[9,10] and the insulin receptor[7], our results will be discussed from this point of view. Liver weight/body weight ratio was significantly higher in Wistar compared to other two strains and only Wistar rats showed considerable amount of GM3 ganglioside in the liver. GM3 ganglioside was primarily described as inhibitor of EGF receptor autophosphorylation[9]. On the contrary, recent investigations with untransformed human fibroblasts, which lacked GM3, and complex gangliosides show that EGF signal transduction is reduced in the absence of GM3[18]. Our results of reduced GM3 content and reduced liver weight/body weight in Lewis and SD rats are consistent with a number of previous studies that have suggested a positive effect of GM3 on cell function. Examples include restoration by GM3 on fibroblast morphology distorted by inhibition of glycolipid synthesis[19] and suppression of metastasis by silencing of GM3 synthase in a tumor model[20]. Pancreas weight/body weight ratio was significantly higher in SD compared to other two strains ($p<0.05$) and pancreatic b-series gangliosides (GD1b and GT1b) were several folds lower expressed in SD compared to Wistar and Lewis rats. After birth, introduction of nutrients into the digestive tract add exogenous factors to the regulation of pancreatic development. Special sensory and gastrointestinal afferent neural signals, along with blood-borne metabolic signals, impinge on parallel central autonomic circuits located in the brainstem and hypothalamus to signal changes in metabolic balance[21]. Taking into account that Wistar and SD rats differ considerably in their mitochondrial respiration, our findings of different hepatic and pancreatic weight/body weight ratios and ganglioside expression are not surprising[22]. The stimulation index of SD pancreatic islets after glucose challenge, (measured on the samples of 20 islets) is the lowest in SD islets compared to that of Wistar and Lewis islets[3]. Reciprocal relation of hepatic and pancreatic weight/body weight ratios in Wistar and SD rats is intriguing because those rat strains differed statistically significantly in weight ratios of both organs in our experiment. In conclusion, our study revealed differences in the hepatic and pancreatic ganglioside phenotypes between Wistar, Lewis and SD rats that could influence growth factor secretion and action.

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GLIKOSFINGOLIPIDNI FENOTIP JETRE I GUŠTERAČE TRIJU NEUROLOŠKI RAZLIČITIH ŠTAKORSKIH SOJEVA

S A Ž E T A K

Tri često upotrebljavana soja laboratorijskih štakora, Wistar, Lewis, Sprague-Dawley (SD), pokazuju različite neurološke karakteristike. Jetra je glavno mjesto sinteze inzulinu sličnog čimbenika rasta (insulin like growth factor, IGF) dok je gušterača mjesto sinteze inzulina. Inzulin potiče neuralni razvoj a utjecaj IGF-I ključan je kod rasta mozga. Glikosfingolipidi (GSL) su važni posrednici u izlučivanju i djelovanju inzulina. U ovoj studiji istraživan je GSL fenotip jetre i gušterače s pretostavkom da se razlikuje između tri štakorska soja. Ukupne GSL frakcije (neutralni i gangliozidi) analizirani su tankoslojnom kromatografijom visokog razlučivanja (high performance thin layer chromatography, HPTLC). Složeni gangliozidi detektirani su imunobojenjem HPTLC ploča B podjedinicom toksina kolere nakon tretiranja neuraminidazom. Štakori Wistar su imali najveći omjer mase jetre i mase tijela a štakori SD najveći omjer mase gušterače i mase tijela. Kod štakora SD pronađena je oskudna količina GD1a i gangliozida b-serije u jetri. B-serija gangliozida također je bila niska u gušterači SD štakora. Ova studija predstavlja razlike u fenotipu jetrenih i gušteračinih gangliozida triju štakorskih sojeva koje bi mogle biti odgovorne za razlike u izlučivanju i djelovanju IGF i inzulina.