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Gender differences in the gene expression profiles of glucose transporter GLUT class I and SGLT in mouse tissues

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Glucose transporters play key roles in controlling blood sugar levels and are recognized as the pharmacological targets of antidiabetic agents. In the present study, we compared the gene expression profiles of glucose transporter GLUT class I and SGLT isoforms in the skeletal muscle, heart, liver, kidney, and brain of male and female mice. The expression profiles of GLUT1-4 and SGLT1-2 in male mouse tissues were similar to those previously reported. Significant gender differences were observed in mRNA expression in terms of individual these glucose transport systems and the tissues examined. Especially, all of the corresponding mRNAs of renal GLUT class I and SGLT isoforms were expressed at higher levels in female mice than in male mice. However, no significant differences were observed in serum glucose concentrations between male and female mice. These results strongly suggest that prominent gender differences exist in the gene expression profiles of these glucose transporters in mouse tissues, and that the quantitative and functional multiplicities of glucose transporters may contribute to the successful regulation of blood glucose concentrations irrespective of gender differences.

1. Introduction

Glucose transport systems have been shown to play essential roles in the homeostatic control of blood glucose concentrations (Zhao et al. 2007). Interest in exploring substances that interact with glucose transport systems as therapeutic agents for diabetes has been increasing (Cuypers et al. 2013). Facilitative and sodium-dependent glucose transport processes are known to be mediated by two distinct families of structurally-related sugar transporters (Zhao et al. 2007). The passive, facilitative transport process is mediated by the family of facilitative glucose transporters (gene symbol SLC2A and protein symbol GLUT). Thirteen members of this family have been identified and categorized into three classes based on sequence similarities: class I (GLUT1-4); class II (GLUT5, 7, 9 and 11); and class III (GLUT6, 8, 10, 12 and 13). Although the roles of the GLUT class I family have been established as glucose transporters in a number of tissues and cell types, there is little information about the class II and class III families. The sodium-dependent glucose transport process is mediated by the Na^+/glucose co-transporter family (gene symbol SLC5A and protein symbol SGLT). To date, at least six members have been classified into this family; however, only SGLT1 and SGLT2 have been characterized in detail.

The expression profiles of GLUT1-4 and SGLT1-2 have already been reported in mammalian male tissues (Zhao et al. 2007). GLUT1 was shown to be ubiquitous in mammalian tissues. GLUT2, SGLT1, and SGLT2 are abundantly expressed in the kidney, and GLUT2 also exists at sufficient levels in the liver. GLUT3 is now considered to be a neuron-specific glucose transporter because it was detected almost exclusively in the brain. The expression of GLUT4 has mainly been observed in the skeletal muscle and heart. However, it currently remains unclear whether gender differences exist in the expression profiles of these glucose transporters. Therefore, we here compared the gene expression levels of GLUT1-4 and SGLT1-2 in the skeletal muscle, heart, liver, kidney, and brain of male and female mice. We also investigated the relationship between blood glucose concentrations and the multiplicity of constitutively expressed glucose transporters with respect to gender differences.

2. Investigations, results and discussion

The profiles of mRNAs for GLUT1-4 and SGLT1-2 in these tissues of male mice were confirmed by real-time PCR (Table 1 and Table 2). GLUT1 was ubiquitous in various tissues in male mice. GLUT2 and GLUT3 were predominantly expressed in the male liver and brain, respectively. The expression of GLUT4 was confined to the skeletal muscle and heart in male mice. The results obtained also confirmed that SGLT1 and SGLT2 were expressed almost exclusively in the kidney, which was consistent with the findings of a previous study (Zhao et al. 2007).

Based on these results, we investigated the expression of these sugar transporters in female tissues under the same experimental conditions (Table 1 and 2). The expression of GLUT4 was

Table 1: Gene expression of GLUT class I in mouse tissues

		GLUT1	GLUT2	GLUT3	GLUT4
Muscle	Male	1.00 ± 0.05	1.00 ± 0.15	1.00 ± 0.14	1.00 ± 0.08 ┐
	Female	1.06 ± 0.07	1.16 ± 0.11	1.06 ± 0.16	0.61 ± 0.03 ┘ **
Heart	Male	0.90± 0.08	2.62 ± 0.37	63.1± 11.9 ┐	90.3±8.94 ┐
	Female	0.99± 0.08	2.20 ± 0.25	14.2±0.75 ┘ **	23.3± 1.47 ┘ ***
Liver	Male	1.70± 0.18	49.0±5.18	9.53 ± 1.06 ┐	ND
	Female	2.05± 0.27	48.8±6.22	0.62 ± 0.17 ┘ ***	ND
Kidney	Male	2.36± 0.27 ┐	12.1 ±0.62 ┐	3.67 ± 0.51 ┐	ND
	Female	5.60±0.90 ┘ **	21.6 ±1.76 ┘ *	6.99 ±0.68 ┘ **	0.367± 0.082
Brain	Male	4.83± 0.64	2.39 ± 0.07 ┐	47558 ± 1243 ┐	ND
	Female	4.69± 0.31	5.63 ± 0.24 ┘ ***	10562 ±1498 ┘ ***	ND

Data were normalized to GAPDH mRNA, which was used as an internal control. Results were expressed relative to the male skeletal muscle group, which were arbitrarily a value of 1, and were shown as the means ± SD of three mice per group. Muscle: skeletal muscle. ND: not detected. Significant differences between male and female groups in each tissue were evaluated by the Student's unpaired *t*-test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, *ver* the respective male group).

lower in female skeletal muscle and heart, while the expression of SGLT2 was higher in female tissues. Furthermore, the expression of GLUT3 in the female heart was significantly lower than that in the males. The expression profiles of glucose transporters in the liver were similar between female and male mice, except for GLUT3, which showed male-dominant expression. The expression of all GLUT class I and SGLT isoforms was higher in the female kidney than in the males. The female-predominant expression of GLUT2 was observed in the brain, while the cerebral expression of GLUT3 was lower in females than in males. These results revealed significant gender differences in the gene expression profiles of these glucose transporters in mice.

Serum glucose concentrations in male and female mice were 20.63 ± 0.54 and 19.91 ± 0.52 mM, respectively. The dietary intakes of male and female mice were 3.72 ± 0.17 and 3.74 ± 0.01 g/day, respectively. No significant differences were observed in serum glucose concentrations and dietary intakes between male and female mice, and this was consistent with the results of a previous study (Cappel et al. 2013). However, gender differences in the gene expression of glucose transporters were observed in various tissues. Therefore, the mechanism underlying the homeostatic control of blood glucose concentrations is likely to differ between males and females; however, glucose concentrations were adequately regulated in both mouse groups. The prevalence of diabetes was previously reported to be lower in females (Wild et al. 2004). GLUT2 has a higher capacity to transport glucose than GLUT1 (Zhao et al. 2007). Previous studies using GLUT2-null mice demonstrated that GLUT2 possessed the function of central glucose sensors, which control feeding (Bady et al. 2006). In the present study, the gene expression level of GLUT2 in the

Table 2: Gene expression of SGLT isoforms in mouse tissues

		SGLT1	SGLT2
Muscle	Male	1.00 ± 0.06	1.00 ± 0.15 ┐
	Female	0.91 ± 0.07	154± 11.9 ┘ ***
Heart	Male	0.15± 0.02	25.2 ± 0.59 ┐
	Female	0.15± 0.03	47.1 ± 7.24 ┘ **
Liver	Male	0.06 ± 0.01	125 ± 13.8
	Female	0.06± 0.01	122 ± 20.1
Kidney	Male	14.0 ± 1.09 ┐	2094 ± 199 ┐
	Female	64.8 ± 5.23 ┘ ***	3524±314 ┘ **
Brain	Male	2.43 ± 0.23	59.5 ± 3.95 ┐
	Female	2.76 ± 0.33	13.7±2.21 ┘ ***

Data were normalized to GAPDH mRNA, which was used as an internal control. Results were expressed relative to the male skeletal muscle group, which were arbitrarily a value of 1, and were shown as the means ± SD of three mice per group. Muscle: skeletal muscle. Significant differences between male and female groups in each tissue were evaluated by the Student's unpaired *t*-test (**: $p < 0.01$; ***: $p < 0.001$, *ver* the respective male group).

female kidney and brain was higher than that in the males. This difference in the expression of GLUT2 may be, at least partly, responsible for the incidence of diabetes in males and females.

Insulin is an important hormone that is used to manage diabetes. Insulin secretagogues and sensitizers have been used to treat diabetes in clinical practice, along with insulin itself (Tilmans et al. 2007). Insulin was previously shown to accelerate the rate of glucose uptake into peripheral tissues from blood vessels. GLUT4 was cloned and functionally characterized as an insulin-responsive glucose transporter (Dobson et al. 1996). The majority of GLUT4 in non-stimulated cells was detected in the intracellular component, and the transporter was redistributed from an intracellular location to the plasma membrane following insulin stimulation. In the present study, the expression of GLUT4 was lower in the female skeletal muscle and heart than in the males. This result supported the findings of a previous study in which females showed a poorer quality of diabetes care than males (Rossi et al. 2013). Inhibitors of SGLT2 have been developed to treat diabetes by suppressing the reabsorption of filtered glucose in the proximal tubules of nephrons (Cuypers et al. 2013). Because the gene expression level of SGLT2 was significantly higher in the female kidney than that in the males, gender differences may exist in the therapeutic efficacy of SGLT2 inhibitors.

A previous study reported that females recovered better from ischemic/reperfusion-induced renal injury than males (Müller et al. 2002). Gender differences in this physiological response may be explained in part by the biochemical mechanism in which sex hormones such as estrogen had a suppressive effect on inflammation and Akt-mediated apoptotic signaling by regulating nitric oxide synthesis (Neugarten et al. 1997). As shown in the present study, female-dominance was observed in the renal expression of GLUT class I as well as SGLT isoforms. Because of the expected higher function of glucose transport in the kidneys of female mice, glucose may be more efficiently supplied to the kidneys of female mice than to those of male mice. This hypothesis provides another reason for the potent resistance of the female kidney to acute ischemic damage.

In conclusion, the present study demonstrated that gender differences existed in the gene expression profiles of GLUT class I and SGLT isoforms in mouse tissues. Therefore, blood glucose concentrations may be successfully regulated in both males and females by the multiple expression of glucose transporters in various tissues. The results of the present study provide a more detailed insight into the physiological and pharmacological roles of glucose transporters.

3. Experimental

3.1. Animals

Male and female B6D2F1 mice aged 5 weeks were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The animals were acclimatized for 7 days before the experiment, and were housed in a clean room maintained at $23 \pm 2\,^{\circ}C$ with a relative humidity of 55 ± 10 % and 12-h light/dark cycle. The consumption of standard rodent chow (5L37, Japan SLC, Inc.) was measured per animal. Biochemical examinations using mouse tissues and serum were conducted on the 7th day, as described below. Experimental protocols and animal care methods in the present study were approved by the Animal Experiment Committee at Osaka Ohtani University.

3.2. Real-time quantitative PCR

The isolation of total RNA and reverse transcription were performed according to our modified method (Nagai et al. 2014), after each tissue had been dissected from animals under anesthesia. cDNA was amplified by PCR at 95 °C for 10 s, at 58 °C (GLUT2 and 3, and GAPDH) or 55 °C (others) for 10 s, and at 72 °C for 30 s. In initial experiments, a melting curve analysis was performed to monitor the purity of the PCR products. Gene expression of the target sequence was normalized relative to the expression of an endogenous control, GAPDH. Synthetic oligonucleotide primers (Hokkaido System Science Co., Ltd., Sapporo, Japan) were designed by Beacon Designer 8 (Bio-Rad Lab. Inc., Berkeley, CA, USA). GLUT1 was amplified with the 5' primer 5'-CCAAGGACACACTAATAC-3' and 3' primer 5'-TAGGAAGAGACAGGAATG-3'. GLUT2 was amplified with the 5' primer 5'-AATTCCTGGCGTCTTCAG-3' and 3' primer 5'-TGTTGATATTTCTAATCGG- AGTCT-3'. GLUT3 was amplified with the 5' primer 5'-TCTACCTTAAGACTT- GAGAACTA-3' and 3' primer 5'-CT TCTAACC- GCTCTTCCA-3'. GLUT4 was amplified with the 5' primer 5'-CGGACCCTA- TACCCTATTCAT-3' and 3' primer 5'-AGGACCAGTGTTCCAGTC-3'. SGLT1 was amplified with the 5' primer 5'-GGTGTAGACTCGGCAACTC-3' and 3' primer 5'-TTCCTCCAGCAAGCCT- ATAATCA-3'. SGLT2 was amplified with the 5' primer 5'-CATAAAGTCGA- GGGTGTT-3' and 3' primer 5'-GGAAGTGACAACCAATCA-3'. GAPDH was amplified with the 5' primer 5'-AAGAAGGTGGTGAAGCAG-3' and 3' primer 5'-TCATACCAGGAAATGAGC-3'. mRNA levels were quantified based on standard curves. Results were expressed relative to the male skeletal muscle group, which were arbitrarily a value of 1.

3.3. Measurement of serum glucose concentrations

Blood was collected by cardiac puncture under anesthesia, and the serum fraction was immediately separated by centrifugation at 4 °C. Glucose concentrations were measured by an enzyme-based colorimetric method using a commercial reagent kit run on a biochemistry analyzer (Spotochem^{TM} EZsp-4430 analyzer; ARKRAY Inc., Kyoto, Japan).

3.4. Statistical analysis

Data were represented as means ± SD. Differences in expression levels in each tissue between male and female mice were compared using the Student's unpaired *t*-test, and differences with a p value of 0.05 or less were considered significant.

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