Journal of Cellular Biochemistry 90:339–346 (2003)

Human Cardiomyocytes Express High Level of Na^+/Glucose Cotransporter 1 (SGLT1)

Lubing Zhou,[1]* Ellen V. Cryan,[1] Michael R. D'Andrea,[2] Stanley Belkowski,[2] Bruce R. Conway,[1] and Keith T. Demarest[1]

[1]Endocrine Therapeutics and Metabolic Disorders Team, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC., Raritan, New Jersey 08869
[2]MorphoMetrics Team, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC., Spring House, PA 19477

Abstract We have quantitatively measured gene expression for the sodium-dependent glucose cotransporters 1 and 2 (SGLT1 and SGLT2) in 23 human tissues using the method of real time PCR. As predicted, our results revealed that the expression of SGLT1 was very high in the small intestine (1.2E + 6 molecules/μg total RNA) relative to that in the kidney (3E + 4 molecules/μg total RNA). Surprisingly, we observed that the expression of SGLT1 in human heart was unexpectedly high (3.4E + 5 molecules/μg total RNA), approximately 10-fold higher than that observed in kidney tissue. DNA sequencing confirmed that the PCR amplified fragment was indeed the human *SGLT1* gene. Moreover, in situ hybridization studies using a digoxigenin (DIG)-labeled antisense cRNA probe corresponding to human SGLT1 cDNA confirm that human cardiomyocytes express SGLT1 mRNA. In contrast, the expression of SGLT2 in human tissues appears to be ubiquitous, with levels ranging from 6.7E + 4 molecules/μg total RNA (in skeletal muscle) to 3.2E + 6 molecules/μg total RNA (in kidney), levels 10–100-fold higher than the expression of SGLT1 in the same tissues. Our finding that human cardiomyocytes express high levels of SGLT1 RNA suggests that SGLT1 may have a functional role in cardiac glucose transport. Since several SGLT inhibitors are currently in development as potential anti-diabetic agents, it may be important to assess the functional consequences of inhibition of SGLT1 in the heart. J. Cell. Biochem. 90: 339–346, 2003.
© 2003 Wiley-Liss, Inc.

Key words: human Na^+/glucose cotransporter 1 and 2 (SGLT1 and SGLT2); gene quantification; in situ hybridization

Absorption of luminal D-glucose in the brush border of small intestine and the proximal tubule of kidney against a concentration gradient occurs via epithelial sodium-dependent glucose cotransporters (SGLTs). At least two major classes of the SGLTs have been described in human tissues thus far: SGLT1 [Lee et al., 1994] and SGLT2 [MacKenzie et al., 1994; You et al., 1995]. The cDNA for SGLT1 was first cloned from rabbit small intestine. The *SGLT1* gene product is a high-affinity, low-capacity transporter with a Na^+/glucose transport ratio of 2:1. In contrast, the *SGLT2* gene product functions as a low-affinity, high-capacity transporter with a Na^+/glucose ratio of 1:1. The cDNA for SGLT2 was first identified in humans [Wells et al., 1992] and subsequently in rats [You et al., 1995] and mice [Tabatabai et al., 2001]. Recently, the cDNA for SGLT3 was isolated from pigs [Kong et al., 1993] and humans [Dunham et al., 1999]. Interestingly, the gene for SGLT3 is located downstream of the *SGLT1* gene and has a similar intron–exon organization, suggesting that it may be an ancient gene duplication [Wright, 2001]. More recently, there is at least one *SGLT* human gene whose function is unknown and at least six orphan cDNAs have been described in addition to the SGLTs of known function [Wright, 2001].

Although the physiological role of SGLT proteins for glucose reabsorption is well documented, very little is known about the distribution of

*Correspondence to: Lubing Zhou, Endocrine Therapeutics and Metabolic Disorders Team, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC., 1000 Route 202, Raritan, NJ 08869.
E-mail: lzhou@prdus.jnj.com

Received 22 May 2003; Accepted 25 June 2003
DOI 10.1002/jcb.10631

© 2003 Wiley-Liss, Inc.

these proteins in non-renal and non-intestinal tissues. Moreover, studies investigating the distribution of these transporters have been limited primarily to the rodents and the pigs [Pajor and Wright, 1991; Veyhl et al., 1993; Lee et al., 1994]. Little is known with regard to the distribution of these genes in other tissues and no detailed information is available with respect to the distribution in human tissues. Since both SGLT1 and SGLT2 play vital roles in absorption of glucose from both the small intestine and the kidney, it is logical to speculate that the inhibitors of SGLT may have medical utilities for the treatment of diabetes. Many pharmaceutical companies have developed compounds to inhibit SGLT-dependent glucose absorption in the kidney and small intestine [Nunoi et al., 2001]. Clearly, the distribution of these transporters may be a critical determinant of safety and tolerability for this emerging class of potential therapeutics.

In this study, we quantified SGLT1 and SGLT2 RNA expression in 23 human tissues using the method of real time PCR. We demonstrated that the expression of SGLT1 was very high in the small intestine and the heart. In contrast, the expression of SGLT2 was ubiquitous in most human tissues. Digoxigenin (DIG)-labeled antisense SGLT1 cRNA probe was used for in situ hybridization and the results revealed that human cardiomyocytes were in fact the cell type expressing SGLT1 in the heart.

MATERIALS AND METHODS

RNA Sources

Human total RNA was purchased from Clontech (Palo Alto, CA), Invitrogen (Carlsbad, CA), and Ambion, Inc. (Austin, TX).

Real Time PCR

The primers used in the real time PCR were designed using the software PrimerExpress (Applied Biosystems, Foster City, CA). The sequences of the TaqMan probe set for human SGLT1 cDNA are: (forward) 5′-CCTCTTCGCC ATTTCTTTCA TC, (reverse) 5′-ATGCACATCC GGAATGGGT, and (TaqMan probe) FAM-CATCGTGGTC ATCTCCCTCC TCACCA-TAMRA. The sequences of the TaqMan probe set for human SGLT2 are: (forward) 5′-TTCAGTCTCC GGCATAGCAA G, (reverse) 5′-CATCTCCATG GCACTCTCTGG, and (TaqMan probe) FAM-AGGAACGGGA GGACCTGGAT GCTG-TAMRA. The amplification fragments are 70 and 108 bp for SGLT1 and SGLT2, respectively. The method used to generate RNA transcripts for SGLT1 and SGLT2 utilized an in vitro transcription reaction, as described previously (Zhou et al., 2001). To generate standard curves, a series of dilutions (from 5E + 7 to 6.4E + 2) of the SGLT1 and SGLT2 RNA transcripts was used in a reaction. Real time PCR was performed in triplicate for each sample in an ABI PRISM 7700 sequence detection system using TaqMan “One-step RT-PCR master mix reagents” (Applied Biosystems). The amount of total RNA used in the reaction was 40 ng and the total volume was 25 μl. The probe set for 18S was purchased from Applied Biosystems and the measurement of human 18S ribosomal RNA served as an internal control for normalization of measuring and loading errors. Real time PCR thermal conditions were: 48°C for 30 min, then 94°C for 5 min, followed by 40 cycles of 94°C for 20 s and 60°C for 1 min. The averages of cycle of threshold (Ct) obtained at the end of real time PCR were plotted against the amount of standard transcripts presented in the PCR reaction to generate the standard curve which was then used for determining the actual molecule numbers of SGLT1 and SGLT2.

Dot Blot Analysis and In Situ Hybridization

Human SGLT1 and SGLT2 full-length cDNA were cloned into the vector of pcDNA3 (Invitrogen) using RT-PCR and conventional recombinant DNA techniques. The cDNA inserts were confirmed by DNA sequencing. Two primers (T7-SGLT1: 5′-AACAAGTAAT ACCACTCACT ATAGGGCAAG AGAGCATCTG AGAAAGA and SP6-SGLT1: 5′-AGCTATTTAG GTGA-CACTAT AGAAGAGATA ATCGTGGGAC AGT) were used to amplify the fragment of human SGLT1 cDNA (GenBank accession number: M24847; 1251–1573 bp) by PCR method using SGLT1-pcDNA3 plasmid as the template. The PCR amplified fragment was gel-purified, validated by restriction enzyme digestion, and used as the template in subsequent in vitro transcription reaction. The DIG-labeled antisense SGLT1-cRNA probe was generated according to the manufacturer’s instructions (DIG RNA labeling kit, Roche Diagnostics, Mannheim, Germany) and quantification of the probe was performed using DIG quantification test strips (Roche Diagnostics). The specificity of probe was verified by dot blot analysis

[Sambrook and Russsell, 2001]. The hybridization of blots and signal detection were performed following the instruction provided by manufacturer (DIG nucleic acid detection kit, Roche Diagnostics).

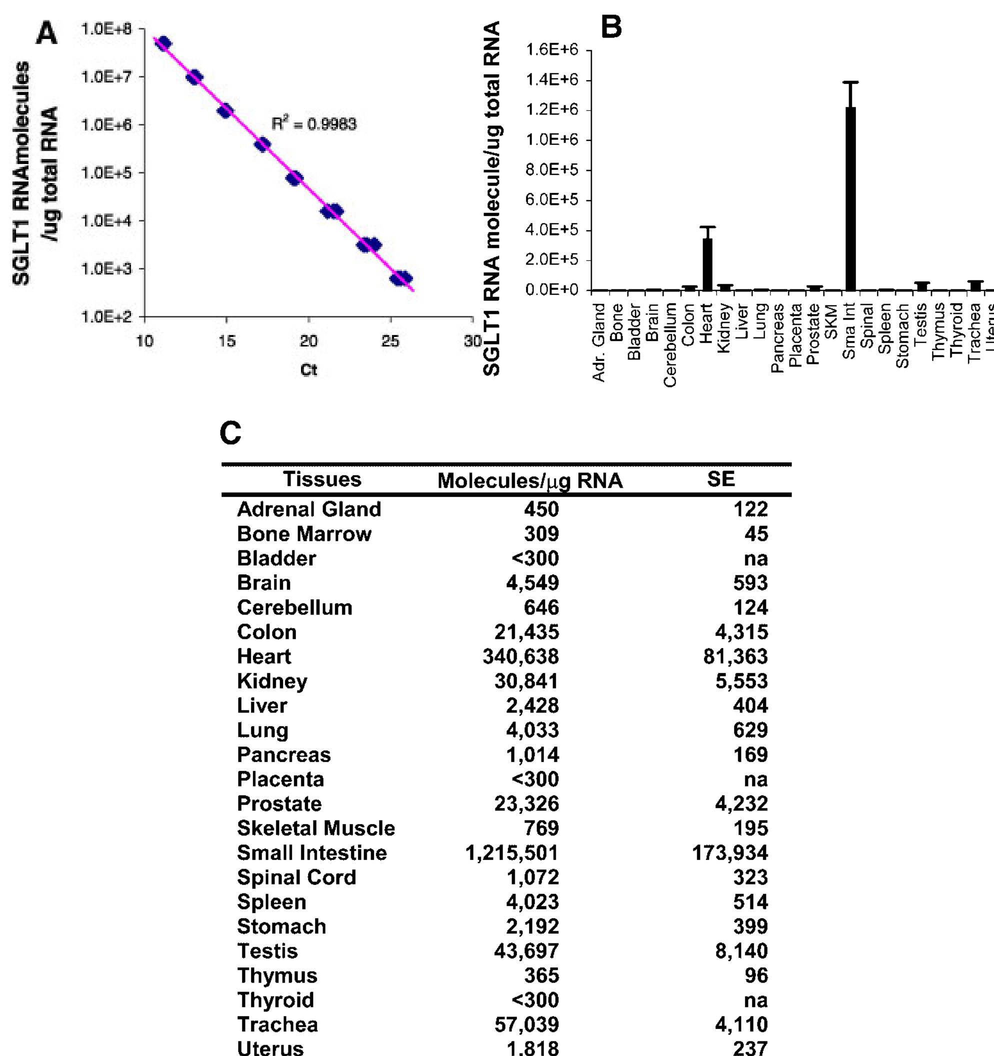
Five-micron tissue sections of human normal heart were mounted onto Plus-superfrost slides (VWR International, Bridgeport, NJ) and then air dried overnight at room temperature. The slides were de-waxed and hydrated with dH_2O. The tissues were digested with pre-diluted pepsin (Invitrogen) for 10 min at 37°C. After washing in dH_2O, the slides were then placed into 3% H_2O_2 for 10 min at room temperature. After a brief wash in dH_2O, the slides were placed into universal buffer (Research Genetics, Huntsville, AL), thoroughly washed, and then dehydrated in 100% alcohol for 1 min. Thirty microlitres of DIG-labeled SGLT1 probe was diluted to 1.0 μg/ml in hybridization buffer (Biomeda, Hayward, CA) and was held in a 42°C water bath. The probe was placed on the slide for 5 min at 95°C on a heat block. The slides were then placed into a humidified chamber and incubated in an oven overnight at 37°C. After the overnight hybridization, the slides were immediately placed into a low stringency wash (2× SSC) for 5 min at 42°C followed by a high stringency wash (0.1× SSC) for 5 min at 42°C. The slides were placed into universal buffer and avidin-horse radish peroxidase-biotin-complex (ABC, Vector Labs, Burlingame, CA) was placed on the tissues for 1 h at room temperature. The slides were washed and exposed to 3,3′-diaminobenzidine for 2×5 min at room temperature. The slides were stained with hematoxylin, dehydrated, cleared in xylene, cover slipped in Permount (Fisher Scientific, Pittsburgh, PA), and then photographed under an Olympus BX50 light microscope.

The positive controls for the in situ hybridization included two biotinylated mRNA probes; one specific to GAPDH mRNA and the other a poly-d(T) probe specific to all mRNA. The negative controls included both the absence of the probe in the probe cocktail and a biotinylated probe specific to LacZ operon mRNA. In addition, we pre-incubated the tissues with RNase A (Sigma, St. Louis, MO) for 2 h at 37°C before hybridization with the SGLT1 probe.

RESULTS

To estimate and compare the expression level of SGLT1 RNA, we measured mRNA from 23 human tissues employing real time PCR method for absolute quantitation. This method provides information on the number of RNA molecules per cell [Zhou et al., 2001]. SGLT1 RNA transcripts made by in vitro transcription were diluted and used to generate the standard curve. The mean values of Ct obtained from the real time PCR were plotted against the amount of RNA transcripts in each of the reactions. The plotted relationship was linear on a log-scale over a 1.0E + 6-fold range and it served as a standard curve for the determination of absolute number of SGLT1 RNA molecules (Fig. 1A). Total RNA isolated from 23 human tissues was simultaneously measured with the diluted SGLT1 RNA transcripts. The mean Ct values of triplicate determinations were normalized to the mean Ct values of ribosomal 18S RNA and then converted to the absolute number of molecules using the standard curve. As expected, small intestine expressed the highest level of SGLT1 mRNA in our quantitative determination. This is in agreement with previous results from rat using Northern blot analysis [Lee et al., 1994]; however, real time PCR method appears to be more sensitive and quantitative. There are approximately 1.2E + 6 of SGLT1 mRNA molecules per 1 μg of small intestine total RNA. Our data revealed that human heart, kidney, colon, testis, trachea, prostate, lung, and liver also express SGLT1 mRNA to varying degrees. The expression levels range from 3.4E + 5 molecules/μg total RNA in the heart to 2.4E + 3 molecules/μg total RNA in the liver (Fig. 1B,C). The lower limit for detection using this assay is approximately 300 molecules/μg total RNA. There was little or no expression of SGLT1 in human bladder, placenta, or thyroid tissues.

Quantitation of SGLT2 mRNA in human tissues was also performed and the results were also in agreement with that previously reported [Wells et al., 1992]. Our results show that SGLT2 mRNA expression is ubiquitous, with the highest level occurring in the kidney. The quantitative measurements demonstrate that there are approximately 3.3E + 7, 1.6E + 7, and 1.0E + 7 molecules of SGLT2 mRNA/μg of total RNA in kidney, small intestine, and testis, respectively (Fig. 2A–C). Skeletal muscle expresses the lowest level of SGLT2 (6.7E + 4/μg of total RNA) among the tissues analyzed. It should be noted that the TaqMan probes were designed to be SGLT subtype-specific.

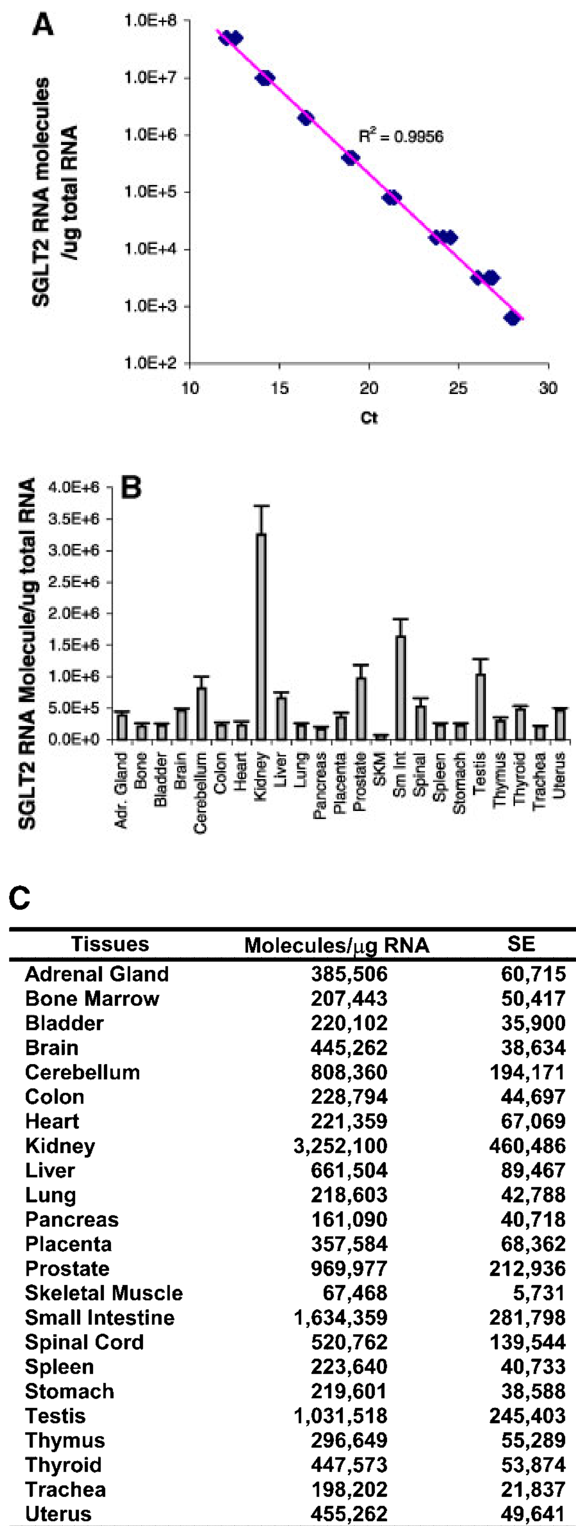


Tissues	Molecules/μg RNA	SE
Adrenal Gland	450	122
Bone Marrow	309	45
Bladder	<300	na
Brain	4,549	593
Cerebellum	646	124
Colon	21,435	4,315
Heart	340,638	81,363
Kidney	30,841	5,553
Liver	2,428	404
Lung	4,033	629
Pancreas	1,014	169
Placenta	<300	na
Prostate	23,326	4,232
Skeletal Muscle	769	195
Small Intestine	1,215,501	173,934
Spinal Cord	1,072	323
Spleen	4,023	514
Stomach	2,192	399
Testis	43,697	8,140
Thymus	365	96
Thyroid	<300	na
Trachea	57,039	4,110
Uterus	1,818	237

Fig. 1. Quantitative analysis of sodium-dependent glucose transporter 1 (SGLT1) RNA. **A**: Standard curve for SGLT1 RNA transcripts. A serial dilution of in vitro transcribed SGLT1 RNA transcripts was reverse transcribed into cDNA and amplified by real time PCR. Cycle threshold (Ct) from the PCR was plotted against the initial RNA copy number of the transcripts. **B**: Expression of SGLT1 RNA in 23 human tissues. Human total RNA was used in the real time PCR. The Ct of PCR was converted into the number of RNA molecules using the standard curve described in A. **C**: Table showing the expression of SGLT1 RNA in human tissues. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Specificity of the individual probe set for SGLT1 and SGLT2 was confirmed in a series of validation studies prior to the quantitation (data not shown). A side-by-side comparison of the expression levels of both *SGLT1* and *SGLT2* genes was possible since we used an absolute quantitation method in the same set of human tissue RNA samples. We found that the expression levels of SGLT2 RNA were generally 10-, 100-, or even 1000-fold higher in the same tissues examined than that of SGLT1 (Figs. 1C and 2C).

The relatively high expression level of SGLT1 mRNA in human heart was somewhat surprising (Fig. 1B,C) as previous data obtained for rat heart showed an undetectable signal using Northern blot analysis [Lee et al., 1994]. To exclude the possibility that our quantitation may have been affected by individual variation, we measured SGLT1 mRNA expression in small intestine, kidney, and heart tissues from two additional individuals. Two separate samples of small intestine, kidney, and heart total RNA were isolated from different individuals and these samples were used in real time PCR quantitation. The results are shown in the Figure 3. Again, small intestine expressed the highest level of SGLT1 mRNA, ranging from 1.4 to



Tissues	Molecules/μg RNA	SE
Adrenal Gland	385,506	60,715
Bone Marrow	207,443	50,417
Bladder	220,102	35,900
Brain	445,262	38,634
Cerebellum	808,360	194,171
Colon	228,794	44,697
Heart	221,359	67,069
Kidney	3,252,100	460,486
Liver	661,504	89,467
Lung	218,603	42,788
Pancreas	161,090	40,718
Placenta	357,584	68,362
Prostate	969,977	212,936
Skeletal Muscle	67,468	5,731
Small Intestine	1,634,359	281,798
Spinal Cord	520,762	139,544
Spleen	223,640	40,733
Stomach	219,601	38,588
Testis	1,031,518	245,403
Thymus	296,649	55,289
Thyroid	447,573	53,874
Trachea	198,202	21,837
Uterus	455,262	49,641

Fig. 2. Quantitative analysis of sodium-dependent glucose transporter 2 (SGLT2) RNA. **A**: Standard curve for SGLT2 RNA transcripts. **B**: Expression of SGLT2 RNA in 23 human tissues. **C**: Table showing the expression of SGLT2 RNA in human tissues. Refer to Figure 1 to know detail. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Tissues	Molecules/μg RNA	SE
Small Intestine-1	2.040,000	52,000
Small Intestine-2	1,370,000	52,500
Heart-1	419,000	12,800
Heart-2	260,000	6,940
Kidney-1	8,760	317
Kidney-2	17,100	520

Fig. 3. Quantitative analysis of SGLT1 RNA from human small intestine, heart, and kidney RNA.

2.0E + 6 molecules/μg total RNA with a mean value of 1.7E + 6 molecules/μg total RNA. The expression of SGLT1 mRNA in kidney ranged from 0.9 to 1.7E + 4 molecules/μg total RNA with a mean value of 1.3E + 4 molecules/μg total RNA. The expression of SGLT1 mRNA in heart, however, ranged from 2.6 to 4.2E + 5 molecules/μg total RNA with a mean value of 3.4E + 5 molecules/μg total RNA. This confirms that the expression level of SGLT1 mRNA in human heart is more than 20-fold higher than that observed in kidney.

To corroborate this unexpected finding, the PCR amplified SGLT1 fragment was sequenced. The results demonstrated that the fragment was part of the human *SGLT1* gene as the sequence was identical to the SGLT1 cDNA (data not shown).

In addition, we performed in situ hybridization studies on normal human tissues of the heart and small intestine to detect SGLT1 mRNA. Human SGLT1 cDNA was used as the template for generating an antisense DIG-labeled cRNA probe and dot blot analysis was used to validate the probe specificity. We demonstrated that the probe was SGLT1-specific since it hybridized only to the SGLT1 RNA transcripts. The probe failed to hybridize to human SGLT2 RNA transcripts or non-specific yeast RNA as shown in Figure 4. The probe was also hybridized to human heart sections (n = 5) using an in situ hybridization method and a representative image is shown (Fig. 5). We detected peri-nuclear labeling of SGLT1 mRNA in cardiomyocytes (brown staining and arrowheads, Fig. 5A). Weakly detected SGLT1 mRNA was also observed to be diffusely distributed in these cell types. We also detected strong SGLT1 mRNA labeling in the small intestine (data not shown), a finding that supports our real time PCR data. Labeling was not observed when the tissue sections were predigested with RNase (Fig. 5B) or when probe diluent was substituted for the probe (data not shown).

Fig. 4. Dot blot analysis. Human SGLT1 cRNA probe labeled with digoxigenin (DIG) was used in the hybridization to show SGLT1 subtype specificity. The top two slots of the panel were loaded with yeast total RNA (50 ng/each slot); the middle two slots were loaded with SGLT1 RNA transcripts (5 and 50 ng/each slot as indicated); the bottom two slots were loaded with SGLT2 RNA transcripts (5 and 50 ng/each slot as indicated).

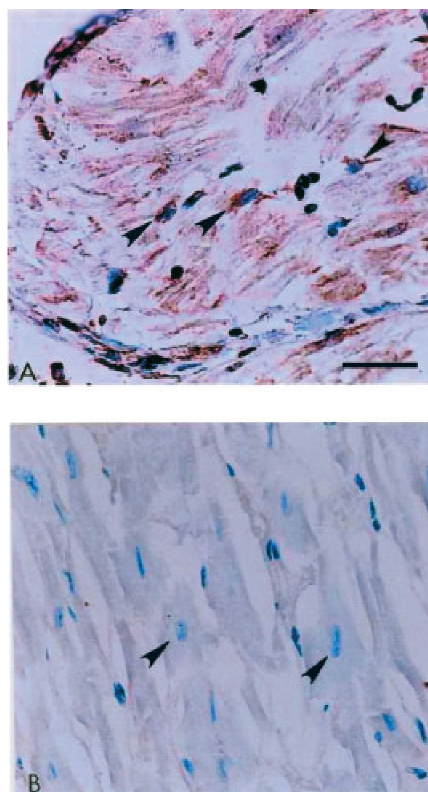


Fig. 5. Results of in situ hybridization: DIG-labeled human SGLT1 cRNA probe was hybridized to normal human heart. **A**: Presence of SGLT1 mRNA in cardiomyocytes of the human heart (brown staining and arrowheads). **B**: Lack of detectable SLGT1 mRNA in cardiomyocytes (arrowheads) after pre-digestion of tissue with RNase. Bar = 25 μm.

DISCUSSION

To date, no comprehensive study of SGLT1 or SGLT2 distribution in human tissues has been reported. Using real time PCR, we have quantitatively measured the expression levels for SGLT1 and SGLT2 RNA in 23 human tissues. Our results show that the small intestine expresses the highest level of SGLT1 and the kidney expresses the highest level of SGLT2. These results are consistent with previous findings [Wells et al., 1992; Lee et al., 1994]. We also demonstrate that the expression of SGLT2 in human is ubiquitous and of generally greater abundance than SGLT1 expression in most of the tissues examined.

Our results also reveal that the human heart expresses SGLT1 mRNA at a relatively high level. Using an SGLT1-specific cRNA probe for in situ hybridization studies, we confirmed that cardiomyocytes were, in fact, the cell-type expressing the *SGLT1* gene. This was surprising considering the earlier observation that little or no SGLT1 mRNA is found in rat hearts [Lee et al., 1994]. There are several possible explanations as to why the expression level of SGLT1 mRNA in heart is low in rat but high in humans. First, we used different techniques for detection. Real time PCR was used in this study and it is generally considered to be more sensitive than Northern blot analysis (see discussion below). A second possible explanation is that the expression level of SGLT1 is different across species. Kanal et al. [1994] failed to detect SGLT1 messages in human kidney using Northern blot analysis but they demonstrated a low level of expression in rat and rabbit kidney. Collectively, these results suggest that the expression of SGLT1 is quite variable across different species.

An SGLT-related protein, SNST1, was described in rabbit heart and kidney [Pajor and Wright, 1991]. Northern blot analysis demonstrated that the expression of SNST1 mRNA was quite high in those tissues. To exclude the possibility that the signal we detected in our real time PCR could be attributed to a human counterpart of SNST1 RNA, we cloned the PCR-amplified fragment and sequenced it.

The result of sequencing indicated that it was human SGLT1 cDNA.

Although numerous studies have demonstrated that facilitative glucose transporters, GLUT1 and GLUT4, are present in the heart, it is not clear how these transporters contribute to glucose delivery under basal conditions. Studies using isolated rat hearts have demonstrated that glucose uptake increases at higher workloads and this increase is accompanied by GLUT4 translocation [Neely et al., 1969; Taegtmeyer et al., 1980]. It was reported that glucose uptake was increased by 2–3-folds during moderate regional ischemia when a canine model was used [Young et al., 1999]. Since GLUT1 is considered to be responsible for basal glucose uptake in many cell types, it is tempting to speculate that GLUT1 is the primary glucose transporter responsible for basal glucose uptake in the heart. The identification of SGLT1 in the cardiomyocyte raises the possibility that basal glucose uptake may be mediated by one or both of these transporters. In any event, further studies will be necessary to elucidate what functional role, if any, the SGLT1 has in myocardial glucose uptake. It will be of particular interest to determine whether or not compensatory changes in SGLT1 expression or activity occur during pathological changes in cardiac function.

Using Northern blot analysis, several groups have characterized the tissue distributions for SGLT1 and SGLT2 in rabbits [Pajor and Wright, 1991], rats [Lee et al., 1994], porcines [Veyhl et al., 1993], and humans [Wells et al., 1992]. Sensitivity of analysis was limited due to the nature of the techniques employed. We have used real time PCR method for the quantitation of SGLT1/SGLT2 messages in human tissues. This method offers several significant advantages. First, it is highly sensitive. In our experiments, we could detect message RNA as low as 300 molecules/μg total RNA. Second, less RNA sample is required. In our experiment, we routinely use 40 ng of total RNA for the quantitation, while at least 10–20 μg of total RNA is usually required for Northern blot analysis. Third, the measurement is performed in a real-time manner during the PCR amplification cycles which permits many samples to be analyzed simultaneously without the concern of reaching an amplification plateau as observed in regular PCR reactions. In our experiments, 23 human RNA were run simultaneously with serial dilutions of standard SGLT1/SGLT2 RNA transcripts and absolute quantitation was obtained from nanogram samples of total RNA.

The functional consequence of SGLT1 expression in the heart remains to be determined. Although much data exists to support a role of glucose absorption in the kidney and small intestine [Kanal et al., 1994; You et al., 1995; Wright, 2001], little is known with regard to SGLT function in different tissues. In an attempt to understand the functional relevance of SGLT1 in the heart, we examined SGLT1 protein expression using several antibodies directed against human SGLT peptides. Unfortunately, none of the antibodies were suitable for the specific detection of SGLT1, even after pre-absorption with human SGLT2 protein. Therefore, the functional significance of SGLT1 in non-renal and non-intestinal tissues remains to be established. Ongoing efforts are underway to generate more specific antibody to analyze SGLT1 protein levels and distributions in tissues.

It has been confirmed that there is a compensatory increase in the amount of glucose flux in diabetic rodent model and this increase correlates well with the increased expression of SGLT1 protein in the cells isolated from the small intestine [Burant et al., 1994]. Since SGLTs play an important role in glucose absorption in the kidney and the small intestine, it is not surprising that several companies are actively pursuing the development of SGLT inhibitors for the treatment of diabetes. Several oral anti-diabetic agents that inhibit SGLT function in kidney and small intestine are being developed [Tsujihara et al., 1996; Oku et al., 1999; Nunoi et al., 2001]. Great efforts are underway to determine the efficacy of such inhibitors in rodents and in human trials. Our finding that human cardiomyocytes express a high level of SGLT1 message provides a piece of significant information for the development of such inhibitors. The important questions as to the functional significance of SGLT1 in the heart and the consequence of SGLT1 inhibition are the subject of ongoing studies.

REFERENCES

Burant CF, Flink S, DePaoli AM, Chen J, Lee WS, Hediger MA, Buse JB, Chang EB. 1994. Small intestine hexose transport in experimental diabetes. J Clin Invest 93: 578–585.

Dunham I, Shimizu N, Roe BA, Chissoe S. 1999. The DNA sequence of human chromosome 22. Nature 402:489–493.

Kanal Y, Lee WS, You G, Brown D, Hediger MA. 1994. The human kidney low affinity Na^+/glucose cotransporter SGLT2. J Clin Invest 93:397–404.

Kong CT, Yet SF, Lever JE. 1993. Cloning and expression of a mammalian Na^+/amino acid cotransporter with sequence similar to Na^+/glucose cotransporters. J Biol Chem 268:1509–1512.

Lee WS, Kanai Y, Wells RG, Hediger MA. 1994. The high affinity Na^+/Glucose cotransporter. J Biol Chem 269: 12032–12039.

MacKenzie B, Panayotobva-heiermann M, Loo DF, Lever JE, Wright EM. 1994. SAAT1 is a low affinity Na^+/glucose cotransporter and not an amino acid transporter. A reinterpretation. J Biol Chem 269:22488–22491.

Neely J, Bowman R, Morgan H. 1969. Effects of ventricular pressure development and palmitate on glucose transport. Am J Physiol 216:804–811.

Nunoi K, Yasuda K, Adachi T, Okamoto Y, Shihara N, Uno M, Tamon A, Suzuki N, Oku A, Tsuda K. 2001. Beneficial effect of T-1095, a selective inhibitor of renal Na^+-glucose cotransporters, on metabolic index and insulin secretion in spontaneously diabetic GK rats. Clin Exp Pharmacol Physiol 29:386–390.

Oku A, Ueta K, Arakawa K, Ishihara T, Nawano M, Kuronuma Y, Matsumoto M, Saito A, Tsujihara K, Anai M, Asano T, Kanai Y, Endou H. 1999. T-1095, an inhibitor of renal Na^+-glucose cotransporters, may provide a novel approach to treating diabetes. Diabetes 48: 1794–1800.

Pajor AM, Wright EM. 1991. Cloning and functional expression of a mammalian Na^+/nucleoside cotransporter. J Biol Chem 267:3557–3560.

Sambrook J, Russsell DW. 2001. Dot and slot hybridization of purified RNA. In: Sambrook J, Russell DW, editors. Molecular Cloning, a laboratory manual. New York: Cold Spring Harbor Lab Press. pp 7.46–7.50.

Tabatabai NM, Blumenthal SS, Lewand DL, Petering DH. 2001. Differential regulation of mouse kidney sodium-dependent transporters mRNA by cadmium. Toxicol Appl Pharmacol 177:163–173.

Taegtmeyer H, Hems R, Krebs H. 1980. Utilization of energy providing substrates in the isolated working rat heart. Biochem J 186:701–711.

Tsujihara K, Hongu M, Saito K, Inamasu M, Arakawa K, Oku A, Matsumoto M. 1996. Na^+-glucose cotransporter inhibitors as antidiabetics: Synthesis and pharmacological properties of 4′-dehydroxyphlorizin derivatives based on a new concept. Chem Pharm Bull 44:1174–1180.

Veyhl M, Spangenberg J, Puschel B, Poppe R, Dekel C, Fritzsch G, Haase W, Koepsell H. 1993. Cloning of a membrane-associated protein which modifies activity and properties of the Na^+-D-glucose cotransporter. J Biol Chem 268:25041–25053.

Wells RG, Pajor AM, Kanai Y, Turk E, Wright EM, Hediger MA. 1992. Cloning of a human kidney cDNA with similarity to the sodium-glucose cotransporter. Am J Physiol 263:F459–F465.

Wright EM. 2001. Renal Na^+-glucose cotransporters. Am J Physiol Renal Physiol 280:F10–F18.

You G, Lee WS, Barros EJ, Kanai Y, Huo TL, Khawaja S, Wells RG, Nigan SK, Hediger MA. 1995. Molecular characteristics of Na^+-coupled glucose transporters in adult and embryonic rat kidney. J Biol Chem 270:29365–29371.

Young LH, Russell RR, Yin R, Caplan MJ, Ren J, Bergeron R, Shulman GI, Sinusas AJ. 1999. Regulation of myocardial glucose uptake and transport during ischemia and energetic stress. Am J Cardiol 83:25H–30H.

Zhou L, Gunnet JW, Moore M, Cryan EV, Xu JZ, Demarest KT. 2001. Human TE671 cells express functional motilin receptors. Biotechnol Letters 23:2067–2073.