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Novel Promoter and Alternate Transcription Start Site of the Human Serotonin Reuptake Transporter (SERT) in Intestinal Mucosa

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Abstract

Selective serotonin-reuptake inhibitors are therapies for psychological and bowel disorders, but produce adverse effects in the non-targeted system. To determine whether human SERT transcripts in the intestine are different from the brain, rapid amplification of cDNA ends, primer extension, and RT-PCR assays were used to evaluate SERT transcripts from each region. Potential slc6a4 gene promoter constructs were evaluated with a secreted alkaline phosphatase reporter assay. A novel transcript of the human slc6a4 gene was discovered that predominates in the intestine, and differs from previous transcripts in the 5' untranslated region. The distinct transcriptional start site and alternate promoter suggest that gastrointestinal SERT can be differentially regulated from brain SERT, may explain why the polymorphism in the previously identified promoter is associated with affective disorders, but not associated with gastrointestinal dysfunction, and suggest the intriguing possibility of the development of site-specific therapeutics for SERT regulation in the treatment of multiple disorders.

Keywords

SLC6A4; 5-HT; SSRI; enteric nervous system; enterochromaffin cell

Serotonin (5-hydroxytryptamine; 5-HT) is a critical signaling molecule in the brain and the gut. In the brain, most 5-HT is synthesized by neurons of the raphe nuclei which send projections throughout the cortex and spinal cord. In the gastrointestinal tract, 5-HT is synthesized by neurons within the enteric nervous system, but the majority of 5-HT is made by enterochromaffin (EC) cells in the mucosal epithelium.¹ Serotonin released from EC cells acts on many nearby targets, including afferent nerve fibers in the lamina propria. Serotonin signaling from neurons and EC cells is terminated by uptake involving the serotonin-selective reuptake transporter (SERT). In the case of neurotransmission, SERT is located on serotonergic nerve terminals, whereas in the intestinal mucosa, all epithelial cells appear to express SERT.

In the presence of serotonin-selective reuptake inhibitors (SSRIs), 5-HT remains in the extracellular space longer, allowing prolonged 5-HT receptor activation. Because the SERT protein is identical in the brain and the gut, systemic SSRIs affect 5-HT signaling both in the brain and the gut. SSRIs are effective therapeutic strategies for both affective disorders and functional bowel disorders,^{2–5} but also have undesired adverse effects in the non-targeted

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system.³ Gastrointestinal symptoms of SSRI treatment, which include nausea and vomiting, diarrhea or constipation, and abdominal pain, can all be explained by enhanced 5-HT signaling in sensory and sensory-motor systems of the gastrointestinal tract.^{6–12}

Given the potential benefit of selectively targeting gut or brain SERT, we sought to determine if SERT regulation in these two regions could be distinguished in the human. SERT is encoded by the 4th member of solute carrier family 6 (*slc6a4*) gene. To date, the human has two isoforms of the transcript that encode SERT, differing in the presence (long) or absence (short) of exon 1b.¹³ A previous report in rodents suggested that the intestinal *slc6a4* transcript involves an alternative transcriptional start site and 5'-untranslated region as compared to the brain transcripts.¹⁴ The human *slc6a4* gene structure differs from its rodent counterparts, having nearly 13kb between exon 1a and exon 1b compared to the approximately 3kb between these exons in rodents.^{13, 14} Likewise, exon 1c of rodents is located in the nearly 9kb region between exon 1b and exon 2, a region that is a mere 737bp in humans.¹⁴ Despite this difference, we hypothesized that the human intestinal *slc6a4* gene differs from the brain transcript in a manner similar to rodents. Here we describe a novel transcript of the *slc6a4* gene that is the major transcript in human intestinal epithelium. Furthermore, we demonstrate that transcription of the SERT gene in intestinal epithelial cells is regulated by a promoter region that is just upstream of the transcriptional start site identified in intestinal cells, but downstream from the previously identified transcriptional start site in neurons.

MATERIALS AND METHODS

Patient Population

Full thickness samples were obtained with consent from the normal margins of resected left colon from three patients (2 male, 1 female; age 60–75) undergoing proctocolectomy for non-obstructive colon carcinoma. Mucosa from this tissue was used to generate complementary DNA (cDNA) samples to be used in all experiments. This protocol was approved by the University of Vermont Institutional Review Board.

Commercial RNA, Genomic DNA and Cell Lines

Total RNA, extracted from human brainstems, was purchased from Ambion (Austin, TX) and used to generate cDNA for subsequent experiments. Human genomic DNA was purchased from Promega (Madison, WI) and used to generate constructs containing potential promoter regions for transcriptional control of the *slc6a4* gene. The immortalized human intestinal mucosal epithelium cell line, Caco-2, was a generous gift from Dr. Jerrold Turner, University of Chicago. These cells express a functional SERT protein.¹⁵ The human choriocarcinoma cell line, JAR, which naturally expresses SERT,¹⁶ was purchased from American Type Culture Collection (Manassas, VA).

RNA extraction and cDNA Synthesis

TRI Reagent (Sigma, St. Louis, MO) was used to extract total RNA from intestinal mucosa and CACO-2 cells. For analysis of the 5' region of the *slc6a4* transcripts, rapid amplification of cDNA ends (RACE)-ready cDNA was generated (SMART System, BD Biosciences). For polymerase chain reaction (PCR), cDNA was synthesized using GeneAmp Gold (Invitrogen). Briefly, in volumes of 20 µl, reverse transcription reactions were initiated with 3 µg total RNA, 6 µg random primers, 1 mM dNTPs, 40 units of RNaseOUT and 200 units of Superscript II Plus Rnase H⁻ reverse transcriptase.

Analysis of 5' untranslated region of the human SERT gene

The 5'-untranslated region of transcripts for the human *slc6a4* gene was analyzed in three ways. First, a 5' rapid amplification of cDNA ends (RACE) reaction was performed using a *slc6a4* gene specific reverse primer (5'-TCTCTATCGTCGGGATTGACACG-3') that corresponds to a sequence in the non-coding region of exon 2. PCR amplification was then performed using a SMART II A oligonucleotide supplied by the manufacturer. Amplified products were separated by agarose gel electrophoresis, isolated, and sequenced by the University of Vermont DNA Analysis Facility of the Vermont Cancer Center.

A second analysis of the 5' region was completed using a primer extension assay. A primer corresponding to a sequence in exon 1b (5'-CGTCGCTCTCCATCCTGCTGGTTAGTAAATGAC-3'), was radioactively end-labeled and used to reverse transcribe RNA from Caco-2 cells and human colon. Reverse transcription was performed using Superscript II (Invitrogen). Primer extension products were electrophoresed on an 8M urea denaturing polyacrylamide sequencing gel. A sequencing reaction was also obtained by using the same end-labeled primer to sequence a plasmid containing the human SERT exon 2 and 5' flanking region, utilizing a sequencing kit (Sequenase version 2; USB, Cleveland, OH). The transcription start site was then identified by running the sequencing reactions alongside the primer extension products. Following electrophoresis, the gel was dried to filter paper and autoradiographed.

A third analysis of the untranslated region of SERT transcripts was completed using RT-PCR. In these analyses, different primer sets were used to amplify products specific for transcripts that contained exon 1c (F:5'-CTTCCCTGCGCCCAGG-3'; R:5'-CAATCCCGACGATAGAGAGC-3') or exon 1a (F:5'-CCAGCCCGGGACCAG-3'; R:5'-ATCCTTGGCAGATGGACATC-3'). In addition, a primer set was generated that corresponded to the published genomic sequence on chromosome 17 that shares identity with the first 53 bp of the rodent exon 1c14 (F:5'-AAGGGCTCAAATTTCTAC-3'; R:5'-ACAGAAGGTTGTTCCACCC-3'). The latter primer set was unable to amplify products from cDNA but could amplify the appropriate 1248 bp product from genomic DNA (positive control; data not shown). PCR reactions, using GeneAmp Gold, were performed using 35 temperature cycles of 95°C for 30s, 68°C for 30s, and 72°C for 1 min, preceded by 95°C for 30s and followed by 72°C for 5 min. Amplified products were separated by agarose gel electrophoresis, isolated and sequenced as described above.

Promoter Analysis with Secreted Alkaline Phosphatase Reporter Assay

The human SERT promoter was amplified from human genomic DNA by PCR (Heruculase, Stratagene), and then subcloned into a TOPO vector for sequencing and used as a template for subsequent constructs. New primers were designed to generate constructs of the SERT promoter from 5' to exon 1A and 5' to exon 2, which were then cloned into the mammalian vector pSEAP-Basic (Clontech Laboratories Inc., CA) containing the promoterless gene for secretory alkaline phosphatase as well as an SV40 polyadenylation signal. Caco-2 and JAR cells were transiently transfected with the *slc6a4* promoter constructs using jetPEI (Polyplus-transfection Inc., New York, NY) or lipofectamine (Invitrogen). Secreted alkaline phosphatase was measured using the Tropix Phospha-Light system (Applied Biosystems, Bedford, MA).

Computational Analysis of Putative Promoters

The sequence of DNA contained in promoters 3, 4 and 5 were analyzed for consensus sequences of transcriptional factor binding sites using PROMO (CNR-ITB, <http://www.itb.cnr.it/sun/webgene/>) and TFSearch (<http://www.rwcp.or.jp/papia/>).

Consensus sequences for binding sites of RNA polymerase, or TATA boxes, were analyzed by Webgene (CNR-ITB, <http://www.itb.cnr.it/sun/webgene/>)

RESULTS

Identification of a novel SERT transcript

A novel transcript of *slc6a4* in the human was identified by rapid amplification of cDNA ends (RACE) assays of human colonic mucosa. Using a gene specific primer within the non-coding region of exon 2 of the *slc6a4* gene, as well as the universal primer that recognizes the 5' SMART oligonucleotide, a PCR product of 327bp was amplified from SMART-cDNA generated from total RNA obtained from the human colonic mucosa. SMART-cDNA generated from human pontine brainstem RNA undergoing the same PCR reaction yielded an amplification product of 161bp (Fig. 1). It was determined that the long product from intestinal RNA corresponded to the previously identified exon 1b (96 bp), with an additional 144 bp upstream of this exon, which we hereafter refer to as exon 1c, spliced to exon 2. The initial 48bp of exon 1c demonstrated a 79% identity to the latter half of exon 1c of rodents. Isolation and sequencing of the 161 bp product from brain RNA revealed a product identical to exon 1a, with the previously identified transcriptional start site¹³ spliced to exon 2, the so-called 'short' transcript.

The same 327 bp product amplified from the human colonic mucosa was amplified from RACE assays of total RNA obtained from Caco-2 cells, an immortalized cell line derived from intestinal epithelium (data not shown). This long product was never identified by RACE in RNA from the human brain, and likewise RNA from the intestine and Caco-2 cells never yielded the 161 bp (brain) product. Primer extension analysis using a primer that complements a region in exon 1b identified the same transcriptional start site as the RACE assay (data not shown).

Additional evidence for this novel transcript and the transcriptional start site comes from RT-PCR reactions with primer sets that amplify transcripts containing either exon 1a or the newly identified exon 1c and contain the coding sequence of exon 2 (Fig. 2). The primer set containing exon 1a (set 1) amplified two products from human brain RNA. The first was that of exon 1a spliced to exon 2, the so-called 'short' transcript. The second product was that of exon 1a, spliced to exon 1b, spliced to exon 2, the so-called 'long' transcript.¹³ This same primer set amplified only the short transcript with RNA extracted from human intestine or Caco-2 cells. Primer set 3, which targeted human exon 1c, amplified a single product with RNA extracted from the brain, intestine and Caco-2 cells (Fig. 2). This is the product that was predicted based on the sequenced product from the RACE assays: exon 1c with exon 1b spliced to exon 2. Because the exon 1c identified in this study did not contain the first half of exon 1c identified from rodents,¹⁴ we used an additional primer set designed to recognize the 53 bp of human genomic DNA (Genbank accession #NC_000017) that has 78% identity with the first 54 bp of the mouse exon 1c. This primer set (set 2) failed to amplify products from RNA extracted from brain, intestine, or Caco-2 cells (Fig. 2).

The combined results of multiple RACE, primer extension and RT-PCR assays were used to construct the sequence of the human exon 1c (see Supplemental Fig. 1). Although this sequence shares 83% identity with the sequence of exon 1c from rodents, no experiments in the current study were able to identify transcription of this region of DNA. Exon 1c lies immediately upstream of the previously identified exon 1b (base pairs 145–241;¹³) and uses the previously identified splice site of exon 1b (two adjacent but separate arrows at position 242) to join exon 2 where the coding sequence is contained. Therefore, the novel transcript identified in this study has 364 base pairs in the 5'-untranslated region. Comparable to the

previously identified short and long transcripts, exon 2 contains the start codon, which begins the translation of the 630 amino acid polypeptide, SERT.

Sequences in intron 1 confer promoter activity in intestinal epithelial cells

Analysis of the 5' untranslated region of the *slc6a4* gene transcript in the intestine indicates that an alternate transcriptional start site is employed in intestinal epithelial cells. Because this putative transcriptional start site is over 12 kb downstream of the previously identified transcriptional start site of this gene,¹³ we hypothesized that the novel transcript would be under the control of an alternate promoter region. Several sequences of human genomic DNA from chromosome 17 were subcloned into the promoterless basic pSEAP vector (schematized in Fig. 3A). These plasmids were then transfected into either Caco-2 cells, or JAR cells, a human choriocarcinoma cell line, and secreted alkaline phosphatase levels were determined as a measure of promoter activity (Figs. 3B and C). In Caco-2 cells, inserts corresponding to regions upstream of exon 1a or 1c demonstrated increased promoter activity. SERT_SEAP construct #1, corresponding to a region 1169 bp upstream of exon 1a, demonstrated the highest activity in this cell line, nearly eight times the activity of the promoterless basic vector. SERT_SEAP constructs corresponding to regions 3237 bp, 1784 bp, and 1211 bp upstream of exon 2, and extending upstream of exon 1c, also demonstrated promoter activity several times greater than the activity of the basic vector. Finally, a construct that shared 760 bp at the 3' end of SERT-SEAP constructs 2–4, and did not extend upstream of exon 1c, demonstrated no significant promoter activity compared to the basic vector. These data suggest that the 588 bp region contained in all three constructs that extended upstream of exon 1c correspond to a minimal promoter region required for expression of exon 1c containing transcripts in Caco-2 cells. In JAR cells, which have been used previously to test *slc6a4* promoters,^{17, 18} the construct extending upstream from exon 1a (#1) demonstrated activity approximately 90 fold that of the promoterless basic vector. The only other construct that demonstrated promoter activity in JAR cells was the construct downstream of exon 1c (#5), which had 5 times the activity of the basic vector. The constructs that corresponded to the region upstream of exon 1C failed to elicit promoter activity in JAR cells.

Because the DNA sequence in SERT_SEAP construct 4 confers promoter activity in Caco-2 cells, whereas the sequence in SERT_SEAP construct 5 does not, we performed computational analysis of these sequences to identify consensus sites for transcription factor binding (see Supplemental Fig. 2). The TATA box sequence predicted 80 bp upstream of exon 1c which is contained in constructs 2, 3 and 4 but not construct 5, is likely the TATA binding protein binding site used to generate *slc6a4* gene transcripts that contain exon 1c. Several transcription factor binding sites were predicted in this region of DNA and may provide enhancer or repressor activity to *slc6a4* gene transcription. The protooncogene products c-Jun and c-Myb have consensus binding sequences near the transcriptional start site, as do the CCAAT/enhancer-binding proteins (C/EBP). It is interesting to note that both c-Myb19 and C/EBP β 20 are necessary for colon epithelium development. The binding sites of several transcription factors involved in inflammatory processes, including glucocorticoid receptors, interferon regulatory factors, signal transducers and activators of transcription (STAT) proteins, and peroxisome proliferator-activated receptors (PPARs) are present in this sequence. These elements may explain the down-regulation of SERT expression during periods of inflammation,^{1, 21} and by exposure of Caco-2 cells to TNF-alpha and interferon-gamma.²² The functional role of these potential response element binding sites must be determined experimentally.

DISCUSSION

The results of this study demonstrate the existence of a novel transcriptional start site and transcript for the human SERT gene. This transcript is the predominant form of SERT mRNA in the intestinal mucosa, and is also expressed in the human brain. The transcript is composed of a distinct 364 bp 5' untranslated region, but contains the same start codon, and thus protein product, as previous reported SERT transcripts.^{13, 23, 24} The novel transcriptional start site is located 144 bp upstream of the beginning of the previously described exon 1b.¹³ The first 48 bp of this transcript share an 81% identity to the latter 47bp (of 101 bp total) of the exon 1c described in mouse.¹⁴ The collective results of several approaches indicate that the human transcriptional start site reported here differs from the start site identified in rodents (which is 53 bp upstream), despite the high homology of these regions of genomic DNA between species. A further difference from the rodent gene is that the human exon 1c-containing transcript continues uninterrupted through exon 1b. There are 96 bp between the end of the region that shares identity with the rodent exon 1c and the beginning of exon 1b that are contained in this novel transcript and have not been described in other species. This difference between humans and rodents is likely necessitated by the difference in gene organization between these species (Fig. 4). In mice, the intron between exon 1a and exon 1b is 2929 bp, and the intron between exon 1b and exon 2 is 8668 bp.¹⁴ Conversely, in humans, the intron between exon 1a and exon 1b is 12,697 bp and the intron between exon 1b and exon 2 is a mere 737 bp.¹³ In mice, the 101 bp exon 1c resides 7563 bp downstream of exon 1b and 1001 bp upstream of exon 2,¹⁴ while in humans exon 1c resides directly upstream of exon 1b.

The novel transcript for human SERT described in this report contains an additional transcriptional start site that differs from those described previously for humans^{13, 23, 24} and rodents.^{13, 25-28} This novel transcriptional start site is flanked by two TATA boxes located 80bp and 244bp upstream and one TATA box located 931bp downstream. The TATA binding protein binding site located 80bp upstream of the start site likely confers the majority of transcriptional activity, but the other sites may also be functional since these constructs maintained transcriptional activity.

Single genes often have multiple transcription start sites under the control of alternative promoters, and these promoters can drive gene expression in tissue-, cell- or developmental stage-specific manners.²⁹ The dominant expression of SERT in the brain, where the predominant transcript contains exon 1a, is by serotonergic neurons.³⁰ The predominance of the SERT transcript containing exon 1c in the intestine is likely explained by the expression of SERT in intestinal epithelial cells.^{21, 31} It is not surprising that cell types that differ as much as epithelial cells and neurons would have distinct mechanisms for controlling transcription of a shared gene. In neurons, SERT acts to terminate the actions of extracellular 5-HT by reuptake of the neurotransmitter back into the neurons that synthesize and release it.³² In the intestinal mucosa, 5-HT is released from enterochromaffin cells in response to mechanical and chemical stimuli, and it is sequestered by epithelial cells, all of which express SERT. Through this efficient termination process, 5-HT can function to coordinate finely orchestrated secretory and motor reflexes.⁹

To compare putative promoter regions for the human *slc6a4* gene, two distinct cell lines that express SERT were transiently transfected with the promoterless pSEAP basic vector into which DNA from the human *slc6a4* gene was cloned. The JAR cell line derived from the human placenta expresses SERT, and has been used for *slc6a4* promoter analysis.^{17, 18} The current study used the human cell lines JAR and Caco-2, which are derived from human intestinal epithelial cells, for *slc6a4* promoter analysis. The SERT_SEAP upstream of exon 1a (#1) was chosen to contain only the positive transcriptional regulatory elements while

avoiding negative regulatory elements, which includes the polymorphic region of variable tandem repeats.^{33, 34} This construct enhanced SEAP activity in Caco-2 cells, albeit far reduced in magnitude as compared to JAR cells. This activity is not surprising as transcripts containing exon 1a were identified in human mucosa and Caco-2 cells, and because the construct lacked negative regulatory elements.³³ We contend however that the major transcript in these cells is the one containing exon 1c, as this was the only product identified by 5'-RACE and primer extension.

Mortensen and colleagues¹⁸ described a sequence in intron 1 that provided modest promoter activity in JAR cells, but not in other cell lines known to express SERT. In the present study, SERT_SEAP construct #s 2, 3, 4 and 5 are all located in intron 1 of the *slc6a4* gene. SERT_SEAP construct #s 2, 3 and 4 all enhanced SEAP activity in Caco-2 cells. SERT_SEAP construct #5 did not provide promoter activity in Caco-2 cells. These data suggest that a novel promoter region for the transcription of the *slc6a4* gene starting with exon 1c is minimally identified as the 588 bp present in construct # 4 but absent in construct #5. The SERT_SEAP construct #5 provided promoter activity in JAR cells perhaps similar to the previous report¹⁸. Surprisingly, however, construct #s 2, 3 and 4 did not enhance SEAP activity compared to the basic construct, all of which contain the sequence in construct #5 in its entirety. This finding suggests that repressive elements that surround the novel transcriptional start site provide additional contributions to cell-type selectivity. The progressively reduced promoter activity, to a level below the promoterless basic construct, as more sequences in intron 1 are added to the constructs, suggests that multiple repressor elements may confer this lack of activity. Perhaps repressive elements upstream of exon 1a¹⁷, not examined in the present study, could confer a similar repressive function in Caco-2 cells. This possibility and the role of the promoter region polymorphisms (see below) deserve further scrutiny using promoter constructs that contain these repressive elements.

The *slc6a4* gene contains two polymorphisms of variable numbers of tandem repeats (VNTR). The first is in the negative regulatory region upstream of exon 1a. The second VNTR is located in the intron between exon 2 and exon 3. Although the downstream polymorphism may act as a regulator of transcription in stem cells,^{35, 36} few linkage and association studies have identified this polymorphism with specific disorders.³⁷ The polymorphic region in the promoter upstream of exon 1a, termed the 5-HTTLPR, has been positively associated with several specific affective disorders and structural differences in the brain.³⁷ The short allele of this polymorphism determines a lower level of transcription *in vitro*,³⁴ but brain imaging studies are inconclusive as to whether this effect translates to less SERT expression *in vivo* (see³⁸). Furthermore, evaluation of SERT RNA levels in human brain stem sections using *in situ* hybridization failed to reveal an association between SERT RNA levels and 5-HTTLPR genotype.³⁹ Association studies of this polymorphic region with disorders of the gastrointestinal tract provide disparate results and meta-analysis of these studies fails to detect an association of 5-HTTLPR with irritable bowel syndrome.⁴⁰ Our finding of intestinal SERT under the control of a novel promoter region and transcriptional start site that is over 13 kb downstream of the 5-HTTLPR suggests that this polymorphic region may have little effect on intestinal epithelial cells that express SERT.

In conclusion, this study demonstrates the existence of a novel transcript of the *slc6a4* gene that encodes SERT and predominates expression in the gastrointestinal tract. This transcript employs an alternate transcriptional start site and expression of this transcript appears to be under the control of a promoter region that is in close proximity to this site. These findings provide a potential mechanistic basis for studies that fail to find a clear association between the 5-HTTLPR and gastrointestinal disorders but do find association with affective disorders despite SERT involvement in both. Functional studies designed to illuminate regulation of

this novel transcript and the possibility of gut-specific SERT-targeted therapies are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

PCR products of 5'-RACE assays on RNA obtained from the pontine region of the human brainstem and the human colonic mucosa separated on an agarose gel. A 161 bp reaction product was amplified from cDNA of the human brainstem, and sequencing confirmed this product was the previously identified exon 1a spliced to exon 2.13 A 327 bp reaction product was amplified from cDNA of the human colonic mucosa. Sequencing of this product identified a novel transcript contain 144 bp immediately upstream of the previously identified exon 1b,13 and exon 1b spliced to exon 2 (see Fig. 3).

Figure 2.

PCR products resulting from the use of three different primer sets on cDNA from the human colonic mucosa (left) and human pontine brainstem (right) separated on an agarose gel. Primer set 1 contained a forward primer corresponding to a sequence in exon 1a and a reverse primer corresponding to a sequence in the coding region of exon 2. Amplification products of 136bp and 233bp correspond to transcripts containing exon 1a spliced to exon 2, and exon 1a spliced to exon 1b spliced to exon 2, respectively. The cDNA of the colonic mucosa amplified a 136bp product but not the 233bp product, while the cDNA from brainstem amplified both products. Primer set 2 contained a forward primer corresponding to 20 bp within the 53bp sequence in the genomic DNA (Genbank accession # NC_000017) upstream of exon 1c that shares identity with the mouse exon 1c14 and a reverse primer corresponding to a sequence in the coding region of exon 2. No amplification products from either the colon or brain samples were detected using this primer set. Primer set 3 contained a forward primer corresponding to a sequence in exon 1c and a reverse primer corresponding to a sequence in exon 2. The sequence of the 250bp amplification product corresponds to the same novel sequence identified by RACE and primer extension. cDNA from both the colon and brain generated this amplification product.

Figure 3.

A) Schematic of the location of promoter construct inserts relative to the human SERT gene used in secreted alkaline phosphatase reporter assays. Promoter activity, as measured by the activity of secreted alkaline phosphatase, of five constructs transfected into Caco-2, human intestinal epithelial cells (B) or JAR, human choriocarcinoma cells (C). Data are the mean ($\pm$ SEM) of four independent experiments with triplicate values for each experiment. Construct 1, which is upstream of exon 1a, demonstrated the highest activity in both Caco-2 cells and JAR cells. In JAR cells, this activity was over 90 times greater than the promoterless basic vector. In Caco-2 cells, this activity was nearly 8 times greater than the promoterless basic vector. Constructs 2, 3, and 4 all contain various lengths of genomic DNA upstream of exon 2, and demonstrated activity in Caco-2 cells that was 5, 6, and 7 times greater than the promoterless basic vector, respectively. Construct 5, however, demonstrated no significant activity above the promoterless basic vector in the Caco cells. Conversely in the JAR cells, constructs 2, 3 and 4 demonstrated no significant activity in comparison to the promoterless vector, while construct 5 had levels of activity 5 fold greater than the promoterless vector. * $P < 0.05$ compared to activity of the promoterless basic vector, ANOVA for repeated measures, with Bonferroni's Multiple Comparisons Test.

Human

Mouse

Figure 4.

Schematic representation of the organizations of the human and mouse *slc6a4* genes and the three known transcripts of each gene that occurs via alternative splicing or alternate transcriptional start sites. Data for the human gene are from the present study while data for the mouse gene are from Ozsarac et al.,¹⁴ and compared to mouse chromosome 11 (Genbank accession # NC_000077). To emphasize the transcribed sequences and to fit within the space provided, exons (shaded boxes) are to scale with other exons but are approximately ten times the scale of introns (solid lines). Introns are in scale to each other. Scales for both exons and introns are the same between the two species. Exons 4–14 for each species are not to scale as these exons are not different between each of the three transcripts for either species.