Human Placental Na\(^+\)-dependent Multivitamin Transporter

CLONING, FUNCTIONAL EXPRESSION, GENE STRUCTURE, AND CHROMOSOMAL LOCALIZATION*

(Received for publication, January 22, 1999)

Haiping Wang‡, Wei Huang‡, You-Jun Fei‡, Hong Xia‡, Teresa L. Yang-Feng‡, Frederick H. Leibach‡, Lawrence D. Devoe§, Vadivel Ganapathy¶, and Puttur D. Prasad¶¶

From the Departments of ‡Obstetrics & Gynecology and ¶Biochemistry & Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912 and the ¶¶Department of Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

We have cloned the human Na\(^+\)-dependent multivitamin transporter (SMVT), which transports the watersoluble vitamins pantothenate, biotin, and lipoate, from a placental choriocarcinoma cell line (JAR). The cDNA codes for a protein of 635 amino acids with 12 transmembrane domains and 4 putative sites for N-linked glycosylation. The human SMVT exhibits a high degree of homology (84% identity and 89% similarity) to the rat counterpart. When expressed in HRPE cells, the cDNA-induced transport process is obligatorily dependent on Na\(^+\) and accepts pantothenate, biotin, and lipoate as substrates. The relationship between the cDNA-specific uptake rate of pantothenate or biotin and Na\(^+\) concentration is sigmoidal with a Na\(^+\):vitamin stoichiometry of 2:1. The human SMVT, when expressed in *Xenopus laevis* oocytes, induces inward currents in the presence of pantothenate, biotin, and lipoate in a Na\(^+\)-, concentration-, and potential-dependent manner. We also report here on the structural organization and chromosomal localization of the human SMVT gene. The SMVT gene is ~14 kilobase pairs in length and consists of 17 exons. The SMVT gene is located on chromosome 2q23 as evidenced by somatic cell hybrid analysis and fluorescence *in situ* hybridization.

Human placenta express a single transport system that transports the micronutrients pantothenate, biotin, and lipoate (1–3). Studies using human placental brush border membrane vesicles (1, 2) and human placental choriocarcinoma cells (JAR) (3) have demonstrated that the stoichiometry of Na\(^+\):substrate for this multivitamin transporter is 2:1, indicating that for every vitamin molecule entering the cell, two Na\(^+\) ions are cotransported into the cell. Since the substrates pantothenate, biotin, and lipoate carry one negative charge at physiological pH, the transport process is electrogenic. Thus the transport system is energized by both the Na\(^+\) gradient as well as the potential difference that exist across the cell membrane. Sodium-dependent pantothenate and biotin transport systems have also been individually characterized in brush border membrane vesicles isolated from the intestine and kidney of rat, rabbit, and human (4–13). Although it is unequivocal that the transport system is Na\(^+\)-dependent, the electrogenercity of the transport system is highly controversial and much debated. It is also not known at this time whether a single transporter, like in placenta, is responsible for the transport of both biotin and pantothenate in the intestine and kidney. Said et al. (6–8), characterizing biotin transport in intestinal brush border membrane vesicles of rat, rabbit, and humans, have shown that the transport process is electroneutral with a Na\(^+\):substrate stoichiometry of 1:1. An electroneutral transport system for biotin has also been reported from rat and human renal brush border membrane vesicles (9, 10). On the other hand, the transport systems for pantothenate and biotin in rabbit renal brush border membrane vesicles have been reported to be stimulated by an inside-negative membrane potential indicating that the transport systems are electrogenic (11–13).

Recently, a cDNA clone encoding the rat sodium-dependent multivitamin transporter (rSMVT)\(^1\) has been isolated from a rat placental cDNA library in our laboratory (14). The cloned cDNA, when expressed in HeLa cells, induces Na\(^+\)-dependent pantothenate and biotin transport activities. The cDNA-specific uptake of both pantothenate and biotin is inhibitable by lipoate. The transporter has a Michaelis-Menten constant (K\(_M\)) of ~5 μM for pantothenate and ~15 μM for biotin. The nucleotide sequence of the cloned transporter predicts a protein of 68.6 kDa with 634 amino acids and 12 potential transmembrane domains. Comparison of the protein sequence of SMVT with other proteins entered in the SwissProt data base shows significant sequence homology with known members of the Na\(^+\)-dependent glucose transporter family.

In this study, we describe the molecular cloning and structural and functional characterization of the human sodium-dependent multivitamin transporter from a placental choriocarcinoma cell line (JAR). This transporter is homologous to the transporter cloned from the rat placenta. The cloned transporter has been characterized by expressing the transporter in human retinal pigment epithelial (HRPE) cells and in *Xenopus laevis* oocytes and by analyzing its transport function by tracer uptake and electrophysiological methods. Northern blot analysis shows that the transcript of hSMVT of 3.2 kb in size is widely expressed in human tissues. The exon-intron organization and chromosomal localization of the human SMVT gene are also presented.

**EXPERIMENTAL PROCEDURES**

*Materials—*The JAR human placental choriocarcinoma cell line was purchased from the American Type Culture Collection (Rockville, MD) and routinely cultured in RPMI 1640 medium supplemented with 10% FBS. The abbreviations used are: rSMVT, rat sodium-dependent multivitamin transporter; hSMVT, human SMVT; HRPE, human retinal pigment epithelium; kbp, kilobase pair; bp, base pair; Mes, 4-morpholineethanesulfonic acid; FISH, fluorescent *in situ* hybridization.

---

\(^{1}\) The abbreviations used are: rSMVT, rat sodium-dependent multivitamin transporter; hSMVT, human SMVT; HRPE, human retinal pigment epithelial; kbp, kilobase pair; bp, base pair; Mes, 4-morpholineethanesulfonic acid; FISH, fluorescent *in situ* hybridization.
fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin as described before (15). The HRPE cell line number 165 originally provided by M. A. Del Monte (W. K. Kellogg Eye Center, Department of Ophthalmology, Ann Arbor, MI) was routinely maintained in Dulbecco’s modified Eagle’s medium-F-12 medium supplemented with 10% fetal bovine serum, 1% penicillin, and 100 μg/ml streptomycin as described before (16, 17). Frogs (Xenopus sp.) were purchased from Nasco (Fort Atkinson, WI), and MEGAscript cRNA synthesis kit was obtained from Ambion (Austin, TX). SuperScript System for cDNA cloning, TRIzol reagent, oligo(dT) cellulose, and Lipofectin were purchased from Life Technologies, Inc. Restriction enzymes were purchased from New England Biolabs (Beverly, MA). Restriction enzyme membranes were purchased from Micron Separations, Inc. (Westborough, MA). d[14C]Pantothenate (51.5 mCi/mmol) and [3H]biotin (58.2 Ci/mmol) were procured from NEN Life Science Products. All of the unlabeled vitamins were purchased from Sigma.

**Screening of the JAR Cell cDNA Library**—The BipDN SIN fragment of the rat placental SMVT cDNA was used as the probe. This fragment was 1.45 kb long and included 75 bp 5′ to the translational start site and most of the coding region. The probe was labeled with [α-32P]dCTP by random priming using the Ready-to-go oligolabeling beads. A JAR cell cDNA library was screened as described earlier (18, 19). Positive colonies were identified and the clones purified by secondary screening.

**DNA Sequencing**—The DNA sequences were determined by the Taq DyeDeoxy terminator cycle sequencing using either [α-35S]dATP or [α-32P]dCTP labeling. The primer extension analysis was performed using the GCG sequences analysis software package GCG version 7.2 (Genetics Computer Group, Inc., Madison, WI).

**Functional Expression in HRPE Cells**—This was done using the vaccinia virus expression system (20) using HRPE cells as described before (14, 21). Subconfluent HRPE cells grown on 24-well plates were first infected with a recombinant (VTF7–3) vaccinia virus encoding T7 polymerase (22). On the following day, oocytes were injected with 50 ng of the vector-cDNA to obtain the cDNA-specific uptake of the vitamins. The restricted fragments were size-fractionated, transferred to nylon filters, and probed with full-length hSMVT cDNA as a probe. The restriction fragments were size-fractionated, transferred to nylon filters, and probed with full-length hSMVT cDNA as a probe. The complete nucleotide sequence of the cloned SMVT gene was determined by digestion with Sall and size-fractionation of the released insert on agarose gel. Digestion of the SMVT clone with XbaI released 3 fragments (1.1, 5.0, and ~9.0 kb) of the genomic DNA insert. The 1.1- and 5.0-kb fragments were subcloned into pSPORT.

**RESULTS AND DISCUSSION**

**Isolation of Human Placental SMVT cDNA from a JAR Cell cDNA Library**—Earlier studies using placental membrane vesicles have demonstrated the presence of SMVT in the brush border membrane of the human placenta (1, 2). We characterized the uptake of pantothenate and biotin in the chorionic villus cell line JAR, and we showed that these cells express a transporter identical to the transporter that is expressed in the placental brush border membrane (3). Unlike RNA isolated from the term placenta that is contaminated with RNA from other tissues like maternal decidua and non-trophoblast cells of the placenta, RNA highly specific to the trophoblast can be isolated from JAR cells in culture. With this in mind, we constructed and screened a JAR cell cDNA library to clone the SMVT cDNA from the recently cloned SMVT as the probe. Upon screening of approximately 3.6 × 10⁸ colonies, several positive colonies were obtained, one of which was arbitrarily chosen for further characterization. The cDNA was designated hSMVT for human sodium-dependent multivitamin transporter.

**Structural Features of hSMVT cDNA**—The hSMVT cDNA is 3162 bp long with an open reading frame of 1908 bp (including the termination codon), encoding a protein of 635 amino acids. The open reading frame is flanked by a 5′-noncoding sequence 391 bp long and a 863-bp long 3′-noncoding sequence (Fig. 1). The cDNA contains a poly(A) tail and a polyadenylation signal (AATAAA) in the 3′-noncoding region. The predicted molecular mass and pI of the protein encoded by the open reading frame are 68.7 and 8.6 kDa, respectively. Preceding the putative initiation codon is the Kozak consensus sequence (GAG GAG TGC). Hydrophobicity analysis of the predicted amino acid sequence using the program of Kyte and Doolittle (29) with a window size of 20 resulted in a hydrophobicity that indicated that the hSMVT protein contains 12 putative transmembrane domains. When modeled with both the amino terminus and the carboxyl terminus of the protein placed on the cytoplasmic side, there are four potential N-linked glycosylation sites (Asn138, Asn488, Asn498, and Asn534), the first site between transmembrane domains 3 and 4 and the other three sites between transmem-
brane domains 11 and 12. The deduced amino acid sequence also displays two sites (Ser 283 and Thr 286) with consensus sequence for protein kinase C-dependent phosphorylation (30, 31) in the cytoplasmic loop between transmembrane domains 6 and 7. Amino acid sequence comparison of hSMVT against rSMVT shows a high degree of homology (84% identity and 89% similarity) between the two proteins indicating that the protein is highly conserved across the species. Blast search (32) against known sequences in the Swiss-Prot data base shows considerable homology with members of the sodium-dependent glucose transporter family confirming that the protein is a member of this family. Among the sodium-dependent glucose transporter family members, the closest relative to SMVT with respect to amino acid sequence similarity is the sodium-dependent iodide transporter (40% identity and 64% similarity) (33).

**Functional Characterization of hSMVT following Expression in HRPE Cells**—The cDNA library used in this study was constructed in pSPORT vector with cDNA inserts unidirectionally ligated in such a way that the sense transcription of the cDNA is under the control of the T7 promoter in the plasmid. This made it possible to employ the transient vaccinia virus expression system for functional characterization of the clone. Initial experiments involving the time course of radiolabeled pantothenate uptake into vector- and cDNA-transfected cells indicated that the uptake of pantothenate was higher in cDNA-transfected cells than in vector-transfected cells. The uptake was also found to be linear up to 60 min, and hence all subsequent experiments were done with a 30-min incubation (data not shown).

The ionic dependence of the cDNA-stimulated pantothenate uptake was investigated by measuring pantothenate transport in empty vector- and cDNA-transfected cells in the presence of various inorganic salts (Table I). Control uptake was measured in the presence of NaCl. Replacement of Na\(^+\) with other cations such as Li\(^+\), K\(^+\), choline, or N-methyl-D-glucamine almost completely abolished the pantothenate uptake in both vector- and cDNA-transfected cells indicating that Na\(^+\) is obligatory for the transport function. Such a marked inhibition was not seen when the Cl\(^-\) in the buffer was replaced with gluconate suggesting the noninvolvement of Cl\(^-\) in the transport process. Replacing Cl\(^-\) with I\(^-\) on the other hand reduced the uptake of the radiolabeled pantothenate by nearly 50%. It is not known at this time whether this inhibition is due to the change in the potential difference across the plasma membrane brought about by the prolonged incubation with NaI or if it is due to I\(^-\) competing with pantothenate for the transport process. The latter hypothesis cannot be ruled out since, as mentioned earlier, hSMVT has very high sequence homology with the Na\(^+\)/I\(^-\) transporter.
cotransporter.

The substrate specificity of the transporter was evaluated by assessing the ability of various unlabeled vitamins and vitamin analogs to inhibit the transport of labeled pantothenate in pSPORT-transfected and pSPORT-cDNA-transfected cells (Table II). The cDNA-specific uptake was determined in these experiments, by subtracting the transport measured in the corresponding empty vector-transfected cells. Pantothenate, biotin, and lipoate were able to inhibit the uptake of radiolabeled pantothenate almost completely. Desthiobiotin, a biotin analog, also inhibited the uptake significantly indicating that the tetrahydrothiophene ring of biotin is not recognized by the transporter. Iminobiotin and diaminobiotin, two other biotin analogs, both of which lack the keto group at the second position of the imidazole ring of biotin, did not inhibit pantothenate uptake indicating that this group is involved in the interaction of biotin with the transporter. The two other water-soluble vitamins tested, namely niacinamide and thiamine, also had no influence on cDNA-induced pantothenate uptake. These results are similar to that obtained in panothenate uptake studies using placental brush border membrane vesicles and JAR choriocarcinoma cells (2, 3).

The kinetics of pantothenate uptake was determined by measuring the rate of pantothenate uptake at varying concentrations of pantothenate (0.25–10 μM) in HRPE cells transfected with the plasmid vector carrying the cDNA insert. Simultaneous measurements at identical concentrations of pantothenate were also done in empty vector-transfected cells. The values obtained were subtracted from the corresponding values of transport measured in cDNA-transfected cells to obtain cDNA-specific uptake. The data obtained were analyzed first by nonlinear regression analysis (Fig. 2A) and confirmed by linear regression (Fig. 2A, inset). The kinetics of biotin uptake by the cloned transporter was also determined similarly by measuring the rate of biotin uptake at varying concentrations of biotin (0.5–20 μM) (Fig. 2B). As can be seen from the figure, the cDNA-induced transport of both biotin and pantothenate is saturable. The experimental data were found to fit best to a model describing the uptake by a single carrier. The linear regression analysis (Eadie-Hofstee transformation) of the data for the cDNA-specific uptake yielded a linear plot for pantothenate and biotin. The kinetic parameters for the carrier-mediated uptake, $K_c$ (Michaelis-Menten constant) and $V_{max}$ (maximal velocity), were 1.5 ± 0.2 μM and 196.0 ± 12.9 pmol/

| Substrate analog | cDNA-specific [14C]pantothenate uptake | Percent control
|------------------|----------------------------------------|------------------|
| NaCl 0.6 | 100 ± 2.6 | 100 ± 2.6 |
| Sodium glutamate | 71.4 ± 6.4 | 71.4 ± 6.4 |
| NaI | 52.5 ± 1.6 | 52.5 ± 1.6 |
| LiCl | 1.8 ± 0.2 | 1.8 ± 0.2 |
| KCl | 0.5 ± 0.1 | 0.5 ± 0.1 |
| Choline Cl | 0.2 ± 0.1 | 0.2 ± 0.1 |
| N-Methyl-d-glucamine Cl | 0.7 ± 0.4 | 0.7 ± 0.4 |

The three physiological substrates of SMVT are pantothenate, biotin, and lipoate. All three substrates possess a long side chain containing a carboxylate group. In addition, biotin has an imidazole- and thiazole-fused ring structure. Three-dimensional structural analysis of the pantothenate molecule indicates that it too exists in a pseudo-ring conformation with a hydrogen bond between the amino group of the β-alanine moiety and the hydroxyl group in the pantoic acid moiety. Lipoate can exist in two states. In its oxidized state, it forms a ring structure by forming a disulfide linkage. In the reduced state with two sulfhydryl groups, lipoate does not have a ring structure. We investigated whether hSMVT can differentiate between these two forms of lipoate. We measured the uptake rate of labeled pantothenate in vector- and cDNA-transfected cells in the presence of varying concentrations of oxidized and reduced lipoate. When reduced lipoate was used, dithiothreitol at a final concentration of 1 mM was used in the buffer to prevent the lipoate from getting oxidized. Dithiothreitol by itself at a concentration of 1 mM had no effect on the uptake of the vitamin (data not shown). The cDNA-specific transport was determined, and the data were analyzed by nonlinear regression analysis (Fig. 3). Both oxidized and reduced lipoate inhibited the uptake of pantothenate; however, the latter was significantly less potent than the former. The IC$_{50}$ values (i.e., the concentration necessary to cause 50% inhibition) for oxidized lipoate was 2.7 ± 0.2 μM and that for reduced lipoate 7.3 ± 0.8 μM. Since the concentration of radiolabeled pantothenate used in the assay was 1 μM and the K$_c$ of hSMVT for pantothenate is 1.5 μM, the K$_c$ value for oxidized lipoate (calculated using the equation $K_c = IC_{50} / (1 + [L/K_c]$ where L is the concentration of pantothenate in the assay) is 1.6 ± 0.2 μM and that for reduced lipoate is 4.4 ± 0.5 μM. Thus, the affinity of hSMVT for oxidized lipoate which has the ring conformation is approximately 3-fold higher than that for the reduced lipoate.

The effect of Na$^+$ on the kinetics of pantothenate and biotin uptake was investigated by measuring the uptake of radiolabeled pantothenate and biotin in HRPE cells transfected with hSMVT cDNA in the presence of varying concentrations of extracellular Na$^+$.

The concentration of NaCl in the extracellular medium was varied from 0 to 140 mM. The cDNA-specific uptake was determined by subtracting the uptake measured with empty vector was subtracted to obtain cDNA-specific uptake. The data represent means ± S.E. for three to six determinations.
Functional Characterization of hSMVT following Expression

**FIG. 2.** Kinetics of pantothenate (A) and biotin (B) uptake induced by hSMVT cDNA in HRPE cells. Transport measurements were made simultaneously in HRPE cells transfected with either empty vector or vector-cDNA construct. Uptake was measured in the presence of NaCl with a 30-min incubation at room temperature. The uptake values obtained in cells transfected with empty vector were subtracted from uptake values obtained in cells transfected with vector containing cDNA. Values represent means ± S.E. for four determinations. Inset, Eadie-Hofstee transformation of the same data. A, concentration of [14C]pantothenate was varied over a range of 0.25–10 μM. B, the concentration of biotin was varied over a range of 0.5–20 μM. Concentration of [3H]biotin was kept constant at 25 nM. For pantothenate, the endogenous uptake observed in cells transfected with empty vector was 20–25% of the uptake measured in cells transfected with plasmid-cDNA construct. For biotin, the corresponding value was 10–15%.

**FIG. 3.** Inhibition of pantothenate uptake by lipoate in HRPE cells transfected with hSMVT cDNA. Uptake of radiolabeled pantothenate was measured in the presence of oxidized lipoate (●) and reduced lipoate (○). Simultaneous measurements were made in cells transfected with plasmid alone and plasmid containing cDNA. The values measured in cells transfected with empty plasmid were subtracted from values measured in cells transfected with plasmid-cDNA construct. The concentration of lipoate was varied from 0.316 to 31.6 μM. 2,3-Dithiothreitol at a concentration of 1 mM was included in the uptake buffer when the effect of reduced lipoate on pantothenate uptake was being measured. Values represent means ± S.E. for four determinations.

in X. laevis Oocytes—A more direct way of characterizing a rheogenic transport system is by electrophysiological means using the two-microelectrode, voltage clamp technique. Since the transport of a substrate molecule across the cell membrane mediated by SMVT is associated with a net transfer of positive charge into cell, the SMVT function should be associated with inward currents under voltage clamp conditions in this experimental approach. The results of the electrophysiological experiments done with SMVT-expressing oocytes are given in Fig. 5. Perfusion of oocytes with buffer containing 10 μM either pantothenate, biotin, or lipoate in the presence of NaCl induced inward currents (~25–35 nA) (Fig. 5A). The inward currents were absent when NaCl in the buffer was replaced with N-methyl-D-glucamine chloride and also in control oocytes injected with water, showing that the substrate-induced currents are dependent on the presence of Na⁺ in the uptake medium and SMVT expression (data not shown). The substrate-induced inward currents were dependent on the testing membrane potential (Fig. 5B). The currents increased in magnitude as the testing membrane potential became more hyperpolarized, demonstrating that the transport activity of SMVT, measured by the magnitude of the substrate-induced inward currents, is stimulated when the membrane potential is made more negative. These data demonstrate unequivocally that SMVT is a potential-sensitive transporter. In addition, since only transportable substrates can induce the inward currents in SMVT-expressing oocytes, the above data also demonstrate for the first time that lipoate is indeed a transportable substrate.

**FIG. 6.** Perifusion of oocytes with buffer containing 10 μM either pantothenate or biotin in the presence of NaCl induced inward currents (~25–35 nA) (Fig. 6A). The current increased in magnitude with increasing hyperpolarization of the testing membrane potential. The substrate-induced currents showed saturation kinetics with respect to pantothenate concentration at different testing membrane potentials (Fig. 6B). The kinetic constants $K_r$ and $I_{max}$ for the transport of pantothenate at different testing membrane potentials were simultaneously in cells transfected with the pSPORT vector. The relationship between the uptake rate and the Na⁺ concentration was sigmoidal (Fig. 4), suggesting the involvement of more than one Na⁺ per pantothenate molecule transported. The data were fit to the Hill equation, and the Hill coefficient, which is the number of Na⁺ ions interacting with the carrier, was calculated. The value was 1.9 ± 0.3 for the uptake of pantothenate (Fig. 4A) and 1.8 ± 0.1 for the uptake of biotin (Fig. 4B). These values were confirmed from the slope of the Hill plots (Fig. 4, insets). This indicates that, for every pantothenate/biotin molecule transported, 2 Na⁺ ions are cotransported. Since both pantothenate and biotin are monovalent anions at physiological pH, the transport process is electrogenic. Thus, both the Na⁺ gradient as well as the difference in the membrane potential across the cell membrane energize the transport process. This greatly increases the concentrative capacity of the transport process.
calculated by nonlinear regression analysis. The kinetic constant $K_t$, a parameter indicative of the affinity of SMVT for the substrate, decreased as the testing membrane potential became more hyperpolarized (Fig. 6C). At a testing membrane potential of $-250$ mV, the $K_t$ was $1.9 \pm 0.4$ nM, whereas the $K_t$ decreased to $1.0 \pm 0.2$ nM when the testing membrane potential was changed to $-2150$ mV. This suggests that the affinity of SMVT for pantothenate increases at hyperpolarizing membrane potentials. The kinetic constant $I_{\text{max}}$, a parameter indicative of the maximal velocity of the SMVT-mediated transport activity, increased as the testing membrane potential became more hyperpolarized (Fig. 6D). The value for $I_{\text{max}}$ increased from $38.7 \pm 2.8$ nA at $-50$ mV to $290.4 \pm 14.8$ nA at $-150$ mV. This shows that SMVT is activated by an inside-negative membrane potential.

Identical experiments were also carried out using biotin and oxidized lipoate as the substrates, and similar results were obtained (data not shown). The affinity constant $K_t$, calculated for biotin and oxidized lipoate, when the transport measure-ments were made in SMVT-expressing oocytes with membrane potential clamped at $-50$ mV, was $19.5 \pm 4.6$ and $7.3 \pm 1.0$ nM, respectively. The affinity constants measured using the electrophysiological technique following expression in *Xenopus* oocytes are significantly higher compared with values obtained using expression in mammalian cells. Although the reason for this is not clear, it may most probably be due to the differences in post-translational processing (e.g. N-linked glycosylation) of the transporter in the two expression systems.

Northern Blot Analysis—Tissue distribution of SMVT mRNA in human tissues was studied using a commercially available membrane blot containing size-fractionated poly(A)$^+$ mRNA from eight tissues: heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (Fig. 7). A 3.2-kbp hybridizing signal was detected in all these tissues. Among the tissues tested, the intensity of the hybridizing signal was the highest in the placenta. Moderate signals were seen in the kidney, liver, and pancreas. The detection of the SMVT-specific transcripts in the kidney in the present study lends credence to the expression of Na$^+$-dependent, electrogenic transport system for pantothenate and biotin in the renal tissue. The fact that the transcripts are detected in all the tissues tested hints at the possible involvement of SMVT in the uptake of pantothenate, biotin, and lipoate in all the tissues.

Isolation of hSMVT Genomic Clone—A genomic clone containing the human SMVT gene was isolated by screening $6 \times$
Characteristics of pantothenate-induced inward currents in hSMVT cRNA injected oocytes. The composition of the uptake buffer was 100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 3 mM Mes/Hepes/Tris, pH 7.5. A, influence of testing membrane potential (V_m) on pantothenate-induced currents (I) at various concentrations of pantothenate. B, influence of pantothenate concentration on pantothenate-induced currents (I) at various testing membrane potentials. C, influence of testing membrane potential on K for pantothenate. D, influence of testing membrane potential on I_max for pantothenate-induced currents.

Structural Features of hSMVT Gene—The SMVT gene is 14.3 kbp in length and contains 17 exons (Fig. 8; GenBank accession number AF116241). The first (184 bp) and the second (67 bp) exons contain only the 5’-noncoding sequence of the cDNA. The second exon is followed by a large intron of 3678 bp. None of the other 15 introns exceed more than 750 bp. Except for the 3rd (532 bp) and 17th exons (989 bp), all exons are less than 200 bp in length. All exon-intron boundaries conform to consensus donor-acceptor sequences (gt/ag) for RNA splicing (Table III).

Chromosomal Localization—We have mapped the chromosomal location of the gene encoding the cloned placental SMVT. Southern blot hybridization of restriction digests of DNA from Chinese hamster-human and mouse-human hybrid cells with the hSMVT cDNA probe showed six human-specific fragments of 4.2, 2.5, 1.5, 1.35, 1.3, and 1.25 kbp (data not shown). Three fragments of 5.5, 2, and 1.6 kbp were detected in DNA of Chinese hamster and four bands of 4, 2.5, 2.2, and 1.6 kbp were present in mouse DNA. All six human-specific bands showed perfect segregation with human chromosome 2. Regional localization of the SMVT gene was carried out by fluorescent in situ hybridization (FISH). The human SMVT genomic clone was used as the probe. In situ hybridization of biotin-labeled genomic clone to human metaphase chromosome spreads corroborated the initial findings of Southern blot analysis on the localization of the SMVT gene to human chromosome 2 (Fig. 9). Twenty metaphase spreads were scored, and each showed hybridization signal on the short arm of chromosome 2, band p23. Although several inherited metabolic disorders have been mapped to this region of the chromosome, none of them appears to be due to a defect in SMVT function. On the other hand, several cases of biotin-responsive multiple carboxylase deficiency have been reported where a defect in the transport of biotin is suspected (34–37). Unlike the more prevalent forms of multiple carboxylase deficiency where the activity of either holocarboxylase synthetase or biotinidase enzyme is invariably low, the activities of both the enzymes are normal in these patients. This disorder is characterized with lactic acidosis and organic aciduria, treatable with pharmacological doses of biotin. Neither the identity of the gene responsible for the biotin-responsive multiple carboxylase deficiency nor its chromosomal localization is known. SMVT may most likely be the defective
In conclusion, we have isolated a cDNA (hSMVT) from a human placental trophoblast cell line cDNA library that, when expressed in HRPE cells, induces Na\(^{+}\)-dependent transport of pantothenate, biotin, and lipoate. The functional characteristics of the induced activity are similar to those of the pantothenate/biotin transport activity described in human placental brush border membrane and in JAR cells. The cDNA has also been expressed in \textit{X. laevis} oocytes and characterized by the electrophysiological technique. The data presented here not only constitute the first direct demonstration of the electrogenic nature of the transport process but also for the first time provide evidence to show that lipoate is a transportable substrate of this transporter. We have also isolated the gene that codes for hSMVT, sequenced it, and characterized the exon-intron organization of the gene. The gene maps to human chromosome 2p23.

REFERENCES
1. Grassl, S. M. (1992) \textit{J. Biol. Chem.} 267, 17760–17765
2. Grassl, S. M. (1992) \textit{J. Biol. Chem.} 267, 22902–22906
3. Prasad, P. D., Ramamoorthy, S., Leibach, F. H., and Ganapathy, V. (1997) \textit{Placenta} 18, 527–533
4. Fenstermacher, D. K., and Rose, R. C. (1986) \textit{Am. J. Physiol.} 250, G155–G160

**TABLE III**

Exon-intron organization of human SMVT gene

| 5’ Exon | Intron | 3’ Exon |
|---------|--------|--------|
| No. | Size | Sequence | Donor No. | Size | Acceptorca |
| bp | bp | Sequence | bp | bp |
| 1 | 184 | CGC CCT CA | gttaacat | 1 | 497 | cttccccag |
| 2 | 67 | CCA CGG AG | gtaaggg | 2 | 3678 | ctttcag |
| 3 | 532 | CCT ATG AG | gtgaagg | 3 | 316 | ataccag |
| 4 | 66 | TTC AGA TG | gtgaagc | 4 | 342 | tgcaacag |
| 5 | 52 | CAA TGC AG | gtgaagg | 5 | 389 | gtccag |
| 6 | 68 | CAG CTC TG | tgaagtgc | 6 | 521 | cctggcag |
| 7 | 155 | GGG TTT GA | tgaagtat | 7 | 418 | ttgcag |
| 8 | 141 | GCT GTC CT | tgaagtgc | 8 | 201 | ttttccag |
| 9 | 130 | CAG ACC AG | sgagaag | 9 | 595 | gttccag |
| 10 | 89 | TCT CTC AG | gtaaccac | 10 | 433 | tttcag |
| 11 | 113 | AGG CCT TG | gtaagggg | 11 | 352 | tccccag |
| 12 | 68 | TGC TGC AG | gtaaatac | 12 | 744 | cccttgg |
| 13 | 87 | ACC CTC CT | gtgaagtg | 13 | 140 | tcggcag |
| 14 | 181 | TCT CCC AA | sgagaag | 14 | 212 | tcctggcag |
| 15 | 104 | ACT CAC TG | gtaagggg | 15 | 236 | ccctccag |
| 16 | 115 | AGC CCC AG | gtaagttc | 16 | 418 | gtttccag |
| 17 | 989 | TTG GAC CT | |

**FIG. 9.** 

**Fluorescent in situ hybridization analysis.** Human metaphase chromosome spreads were hybridized to biotin-labeled hSMVT genomic clone. A representative chromosome spread is shown. Hybridization signals are indicated by arrows.

**FIG. 8.** 

**Exon-intron organization of the human SMVT gene and its organizational relationship to the hSMVT cDNA.** Black boxes in the gene represent the protein-coding regions of the exons, and open boxes represent 5’-noncoding region in exons 1, 2, and 3 and 3’-noncoding region in exon 17. The numbers in cDNA represent the position of the first nucleotide of each exon.
Human Placental Sodium-dependent Multivitamin Transporter

5. Karnitz, L. M., Gross, C. J., and Henderson, L. M. (1984) Biochim. Biophys. Acta 769, 486–492
6. Said, H. M., and Redha, R. (1987) Am. J. Physiol. 253, G631–G636
7. Said, H. M., and Mohammadkhani, R. (1992) Biochim. Biophys. Acta 1107, 238–244
8. Said, H. M., Redha, R., and Nylander, W. (1987) Am. J. Physiol. 253, G52–G55
9. Baur, B., Wick, H., and Baumgartner, E. R. (1990) Am. J. Physiol. 258, F840–F847
10. Baur, B., and Baumgartner, E. R. (1993) Pfluegers Arch. 422, 499–505
11. Barbarat, B., and Podevin, R. A. (1986) J. Biol. Chem. 261, 14455–14460
12. Podevin, R. A., and Barbarat, B. (1986) Biochim. Biophys. Acta 856, 471–481
13. Barbarat, B., Chambrey, R., and Podevin, R. A. (1991) J. Physiol. (Lond.) 443, 79–90
14. Prasad, P. D., Wang, H., Kekuda, R., Fujita, T., Fei, Y. J., Devoe, L. D., Leibach, F. H., and Ganapathy, V. (1998) J. Biol. Chem. 273, 7501–7506
15. Kishimoto, A., Nishiyama, K., Nakanishi, H., Urazuji, Y., Nomura, H., Takeyama, Y., and Nishizuka, Y. (1985) J. Biol. Chem. 260, 12492–12499
16. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
17. Smanik, P. A., Liu, Q., Furminger, T. L., Ryu, K., Xing, S., Mazaferri, E. L., and Jhiang, S. M. (1996) Biochem. Biophys. Res. Commun. 226, 339–345
18. Thoene, J. G., and Baker, H. (1983) N. Engl. J. Med. 308, 639–642
19. Thoene, J. G., Lemons, R., and Baker, H. (1983) N. Engl. J. Med. 308, 639–642
20. Blakely, R. D., Clark, J. A., Rudnick, G., and Amara, S. G. (1991) Anal. Biochem. 194, 302–308
21. Wu, X., Keen, E., Huang, W., Fei, Y. J., Leibach, F. H., Chen, J., Conway, S. J., and Ganapathy, V. (1998) J. Biol. Chem. 273, 32776–32786
22. Parent, L. S., Supplisson, S., Loo, D. D. F. and Wright, E. M. (1992) J. Membr. Biol. 125, 49–62
23. Loo, D. D. F., Hazama, A., Supplisson, S., Turk, E., and Wright, E. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5767–5771
24. Mackenzie, B., Loo, D. D. F., Fei, Y. J., Liu, W. J., Ganapathy, V., Leibach, F. H., and Wright, E. M. (1996) J. Biol. Chem. 271, 5430–5437
25. Mackenzie, B., Fei, Y. J., Ganapathy, V., and Leibach, F. H. (1996) Biochim. Biophys. Acta 1284, 125–128
26. Prasad, P. D., Li, H. W., Fei, Y. J., Ganapathy, M. E., Fujita, T., Plumley, L. H., Yang-Feng, T. L., Leibach, F. H., and Ganapathy, V. (1998) J. Neurochem. 70, 443–451
27. Shaper, N. L., Lin, S. P., Joziasse, D. H., Kim, D. Y., and Yang-Feng, T. L. (1991) Genomics 12, 613–615
28. Kosak, M. (1986) Cell 44, 283–289
29. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
30. Woodget, J. R., Gould, K. L., and Hunter, T. (1986) Eur. J. Biochem. 161, 177–184
31. Kishimoto, A., Nishiyama, K., Nakanishi, H., Urazuji, Y., Nomura, H., Takeyama, Y., and Nishizuka, Y. (1985) J. Biol. Chem. 260, 12492–12499
32. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
33. Smanik, P. A., Liu, Q., Furminger, T. L., Ryu, K., Xing, S., Mazaferri, E. L., and Jhiang, S. M. (1996) Biochem. Biophys. Res. Commun. 226, 339–345
34. Blakely, R. D., Clark, J. A., Rudnick, G., and Amara, S. G. (1991) Anal. Biochem. 194, 302–308
35. Wu, X., Keen, E., Huang, W., Fei, Y. J., Leibach, F. H., Chen, J., Conway, S. J., and Ganapathy, V. (1998) J. Biol. Chem. 273, 32776–32786