(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 March 2002 (07.03.2002)

PCT

(10) International Publication Number
WO 02/18541 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/41986

(22) International Filing Date:
4 September 2001 (04.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/229,929 1 September 2000 (01.09.2000) US

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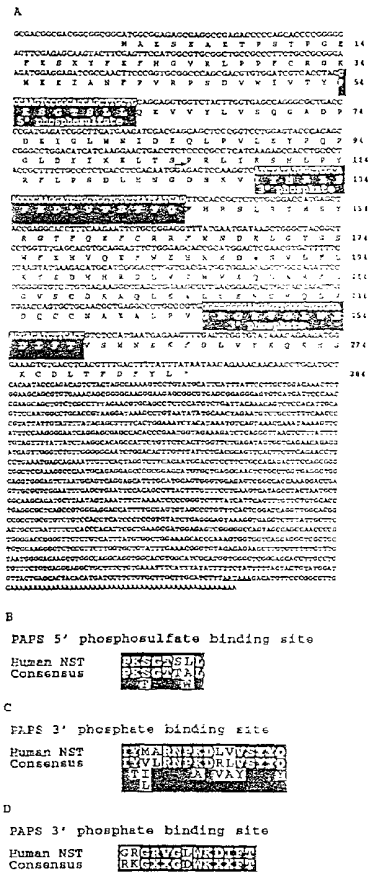
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: A NEURAL SPECIFIC CYSTOSOLIC SULFOTRANSFERASE FOR DRUG SCREENING



(57) Abstract: SULTn, a gene encoding a novel nervous system sulfotransferase, has been identified by searching an expressed sequence tag database for sulfotransferase specifically expressed in the brain. SULTn is a polypeptide of 33,085 Mr which structurally resembles cystolic sulfotransferases (i.e., 34-39% identity), with signature 3'-phosphoadenosine 5'-phosphosulfate cosubstrate binding-site motifs within the polypeptide's catalytic domain. SULTn binds to adenosine 3',5'-diphosphate-agarose, an analogue of the cosubstrate and competition experiments demonstrated the specificity of this binding. Unlike all konwn cystolic sulfotransferases, which are highly expressed in the liver, human SULTn transcripts of 2.4 kb were detected by Northern blot analysis only in the adult brain, and not in liver, heart, placenta, lung, skeletal muscle, kidney, or pancreas. In situ hybridization of rat embryos revealed early developmental expression of the SULTn gene in the central, peripheral and enteric nervous systems. SULTn is widely expressed in the adult nervous system including the Purkinje cell layer of the cerebellum. The in situ hybridization pattern in the nervous system was consistent with the staining of neurons, and was dissimilar to the hybridization of glial fibrillary acidic protein, an astrocyte marker. The human SULTn gene has a toatl of 7 coding exons on chromosome 22 (22q13) that span greater than 47 kb of genomic DNA. Thus, the identification of a novel cystosolic sulfotransferase that is expressed exclusively in nervous tissue reveals the existence of nervous system-specific sulfonation.

WO 02/18541 A2

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A NEURAL SPECIFIC CYSTOSOLIC SULFOTRANSFERASE FOR DRUG SCREENING

Government Support

The present invention was made with Government Support under Contract Numbers MH49469, CHD22539, and AA11697 awarded by the National Institutes of Health. Accordingly, the Government may have certain rights in the invention.

Field of the Invention

The present invention relates generally to recombinant engineering and protein chemistry techniques. More specifically, the present invention relates to the expression of novel nervous system proteins and elucidation of their biological function.

Background of the Invention

Pharmacogenetics is the study of the role of inheritance in variation of drug response, a variation that often results from individual differences in drug metabolism. Sulfation is an important pathway in the metabolism of many neurotransmitters, hormones, drugs, and other xenobiotics. Sulfate conjugation is catalyzed by members of a gene superfamily of sulfotransferase enzymes which have, by convention, been assigned the abbreviated designation "SULT". Sulfotransferases have also been designated as "PSTs" in the literature. Included among the nine cytosolic SULTs presently known to be expressed in human tissues are three phenol SULTs, SULT1A1, 1A2 and 1A3, which catalyze the sulfate conjugation of many phenolic drugs and other xenobiotics.

Sulfotransferase is an important component of nervous system function as a result of its being able to alter the biological activity of various signaling molecules. *See, e.g.*, Roth, *Trends Pharm. Sci.* 7: 404-407 (1986); Falany, *FASEB J.* 11: 1-2 (1997); Weinshilboum, *et al.*, *FASEB J.* 11: 3-14 (1997). Regulation by sulfotransferases results in activation or inactivation of biologically potent endogenous substrates including, but not limited to: catecholamine and peptide neurotransmitters, steroid hormones, neurosteroids and proteins. For example, sulfonation of the neurotransmitter cholecystokinin is crucial for binding to its receptor. *See, e.g.*, Gigoux, *et al.*, *J. Biol. Chem.* 273: 14380-14386 (1998). In addition, monoamine-sulfating phenol sulfotransferase has been shown to inactivate dopamine to form dopamine sulfate, the major form of dopamine in the human circulation. *See, e.g.*, Doousa and Tyce, *Proc. Soc. Exp. Biol. Med.* 188: 427-434 (1988). Moreover, the neuroactive steroids pregnenolone sulfate and dehydroepiandrosterone sulfate are also able to exert direct modulatory effects on neurotransmitter receptors. *See, e.g.*, Wu, *et al.*, *Mol. Pharmacol.* 40: 333-336 (1991); Farb and Gibbs, in: *CNS Transmitters and Neuromodulators* CRC Press, New York, NY, pp. 23-36 (1996); Weaver, *et al.*, *Brain Res.* 803: 153-160 (1998); Yaghoubi, *et al.*, *Brain Res.* 803: 153-160 (1998); Park-Chung, *et al.*, *Brain Res.* 830: 72-87 (1999). Pregnenolone sulfate enhances learning and memory and is implicated in playing a role in cognition. *See, e.g.*, Mayo, *et al.*, *Brain Res.* 607: 324-328 (1993); Vallee, *et*

al., Proc. Natl. Acad. Sci. U.S.A. 94: 14865-14870 (1997).

Two structurally-distinct types of sulfotransferases have been demonstrated to exist, localized in either the cytosol or Golgi, which act on different substrates. The substrates of cytosolic sulfotransferases are steroids and synthetic and endogenous chemicals, whereas the sulfotransferases of the trans-Golgi network are integral membrane glycoproteins that act on newly-synthesized proteins transiting the secretory pathway. Both types of sulfotransferases transfer the sulfur trioxide sulfonate group from the donor 3'-phosphoadenosine 5'-phosphosulfate. The acceptor groups are aromatic or aliphatic hydroxyls whose conjugation gives rise to a sulfate group. A Golgi-associated sulfotransferase that is exclusively expressed in the nervous system and implicated in modifying and maintaining synaptic specializations has recently been described. *See,* Nastuk, *et al., J. Neurosci.* 18: 7167-7177 (1998). However, the cytosolic sulfotransferases have been studied mainly with regard to their role outside the nervous system, especially in the liver. *See, e.g.,* Falany, *FASEB J.* 11: 1-2 (1997); Weinshilboum, *et al., FASEB J.* 11: 3-14 (1997); Dunn and Klaassen, *Drug Metabol. Disposition* 26: 598-604 (1998).

Thus, in conclusion, a cytosolic sulfotransferase that is exclusively-expressed in the nervous system where it serves an important functional role by catalyzing the

formation of a sulfate group on small neurochemicals has, prior to the present invention, not yet been identified.

Summary of the Invention

In brief, a novel sulfotransferase is disclosed that, unlike other known cytosolic sulfotransferases, was not detected in the liver, kidney, or pancreas, but was found to be exclusively-expressed in the nervous system. This novel nervous system-specific sulfotransferase has been designated herein as "SULT". In the present invention, the cDNA and genomic sequences of this novel sulfotransferase have been characterized, and the binding of the recombinant SULTn polypeptide to PAP-agarose and its expression in the embryonic and adult nervous system have been examined.

The SULTn transcripts were demonstrated to be abundantly expressed in an identified neuronal cell type (*i.e.*, the Purkinje cells of the cerebellum) and are also widely, but discretely localized, within the adult brain. SULTn was also found to be expressed in the developing embryo in all three identified branches of the mammalian nervous system (*i.e.*, the central (CNS), peripheral (PNS), and enteric nervous systems).

Description of the Figures

FIG. 1: illustrates potential PAPS co-substrate binding sites in the SULTn polypeptide.

Panel A: illustrates the 2419 nucleotide sequence of human SULTn [SEQ ID NO:1] and the inferred amino

acid sequence [SEQ ID NO:2]. The amino acids with homology to the putative PAPS co-substrate binding sites are shown, in a black box. The locations of the PAPS 5'-phosphosulfate (5'-phosphosulphate) binding site and two 3'-phosphate binding sites are indicated. The polyadenylation signal is underlined. The numbers to the right of the translation represent the amino acid number in the human SULTn polypeptide. The sequences of the human and rat SULTn cDNA were deposited in GenBank, with Accession Numbers AF176342 and AF176343, respectively. An arrow indicates the start of the partial rat SULTn sequence that has been deposited in GeneBank, but is not shown. Lowercase letters indicate the human SULTn amino acids that are not conserved in rat SULTn.

Panel B: illustrates the 5'-phosphosulfate binding site of human SULTn aligned with the consensus sequence of cytosolic and membrane-bound sulfotransferases. The identical amino acid residues are indicated in black and differences in gray. Where there is an alternative amino acid in the consensus, it is shown below.

Panel C: illustrates the 3'-phosphate binding sites of human SULTn aligned with the consensus sequence of membrane-bound sulfotransferases.

Panel D: illustrates the 3'-phosphate binding sites of human SULTn aligned with the consensus sequence of cytosolic sulfotransferases. In the consensus

sequence for cytosolic sulfotransferases, the "X" indicates any amino acid residue may be found at that position.

FIG. 2: illustrates that the amino acid alignment of SULTn indicates that it is a member of the cytosolic sulfotransferase gene family. The amino acid sequence of the human nervous system expressed sulfotransferase (SULTn) was aligned with human estrogen sulfotransferase (SULT1E/EST; GenBank Accession No. S77383); monoamine-sulfating phenol sulfotransferase (SULT1A3/PST; GenBank Accession No. X84653); and dehydroepiandrosterone sulfotransferase (SULT2A/DHEAST; GenBank Accession No. X70222), using the Genetics Computer Group program pileup. The amino acid residue numbers are indicated on the right. Black and gray boxes indicate identical and similar amino acid residues, respectively. A gray box indicates the location of the 5'-phosphosulfate and 3'-phosphate binding sites. The amino acid residues encoded by the 127 bp and 95 bp exon are indicated by a black box below the amino acid sequence.

FIG. 3: illustrates the human SULTn genomic structure. A comparison of the gene structure of human SULTn and other cytosolic sulfotransferase gene family members reveals two exons of identical size. The gene structure of human SULTn is compared to human SULT1E/EST and human SULT2A/DHEAST. Filled and open boxes represent the coding and non-coding regions of exons, respectively. The introns are shown as lines with breaks (-//-) to indicate that the introns are not drawn to scale. The

number of each exon is indicated above the box and the number of bases in the exon is shown below the box. A gray box highlights the two exons in SULTn, SULT1E, and SULT2A, with an identical number of nucleotide. The approximate total size of each gene is indicated on the left in kilobases (kb).

Detailed Description of the Invention

I. Definitions

Amino Acids: Amino acids are shown herein either by three letter or one letter abbreviations as follows: A Ala (Alanine); C Cys (Cysteine); D Asp (Aspartic acid); E Glu (Glutamic acid); F Phe (Phenylalanine); G Gly Glycine; H His (Histidine); I Ile Isoleucine; K Lys (Lysine); L Leu (Leucine); M Met (Methionine); N Asn (Asparagine); P Pro (Proline); Q Gln (Glutamine); R Arg (Arginine); S Ser (Serine); T Thr (Threonine); V Val (Valine); W Trp (Tryptophan); Y Tyr (Tyrosine).

Antigen: A substance recognized by T- and B-cell receptors and which activates lymphocytes by interacting with the combining sites of T- or B-cell receptors.

Biological Activity: The capacity of acting or reacting in some specific way in a biological system.

Cloning Vector: A plasmid, phage DNA, cosmid, or other DNA sequence which is able to replicate within a host cell, which is characterized by one or more restriction endonuclease recognition site at which such DNA sequences may be cut in a determinable fashion without attendant loss of an essential biological

function of the DNA (*e.g.*, replication, production of coat proteins or loss of promoter or binding sites) and which contain a marker suitable for use in the identification of transformed cells (*e.g.*, antibiotic resistance or bacterial colony color selection). A cloning vehicle is often called a vector.

DNA Sequence: A linear array of nucleotides connected one to the other by phosphodiester bonds between the 3'- and 5'-carbons of adjacent pentoses.

Expression: The process by which a polypeptide is produced by a gene or DNA sequence. It is a combination of transcription and translation. As it relates to proteins, expression refers to the directed synthesis of large amounts of desired proteins. Overexpression refers to production of a desired protein in amounts exceeding, that which is normally produced by the cell in question.

Expression System: As utilized herein, the term "Expression System" is comprised of all the elements (*e.g.*, expression vector, insert nucleic acid, promoters, termination sequences, polymerases, host cells, and the like) which are required for the transcription and subsequent translation of a protein of interest from an expression vector construct which has been introduced into a host cell.

Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is

called a nucleoside. The base characterizes the nucleotide. The four DNA bases are adenine (A), guanine (G), cytosine (C), and thymine (T). The four RNA bases are A, G, C, and uracil (U).

Nucleotide Base Sequence: A linear array of nucleotides in a DNA molecule commonly prepared from four dNTP precursors: dATP, dCTP, dGTP and dTTP. Modified bases, other than the usual four found in DNA, may be incorporated.

Peptide: A compound consisting of naturally-occurring or synthetic amino acids which also can be further modified, as described above, which is covalently linked through peptide bonds formed from the carboxyl group of one amino acid and the amino group of the next amino acid by elimination of a molecule of water. The amino acids can be either naturally occurring amino acids as described above, chemically synthesized variants of such amino acids such as norleucine, or modified forms of these amino acids which can be altered from their basic chemical structure by addition of other chemical groups which can be found to be covalently attached to them in naturally occurring compounds. Some of the modifications so attached include: phosphate groups, lipid groups, nucleotide groups, and polymers of sugars and will include other modifications known to those skilled in the art.

Plasmid: A non-chromosomal, double-stranded DNA sequence comprising an intact "replicon" such that the plasmid is replicated in a host cell. When the plasmid

is placed within a unicellular organism, the characteristics of that organism may be changed or transformed as a result of the DNA of the plasmid. For example, a plasmid carrying the gene for tetracycline resistance transforms a cell previously sensitive to tetracycline into one, which is resistant to it. A host cell transformed by a plasmid is designated a "transformant".

Recombinant DNA Molecule: A molecule consisting of segments of DNA from different sources which have been joined end-to-end outside of living cells and are able to be maintained in living cells.

In brief, a bioinfomatics-based approach was used to search for homologs of cytosolic sulfotransferases that are expressed in the nervous system. The novel sulfotransferase of the present invention is unlike other known cytosolic sulfotransferases, in that it is not detected in the liver, kidney, or pancreas, but rather is exclusively expressed in the nervous system. The cDNA and genomic sequences of this novel sulfotransferase have been characterized, and the binding of the recombinant protein to PAP-agarose, and studying its expression in the embryonic and adult nervous system has also been examined.

Sulfonation is an important part of neuronal function that influences learning, memory and cognition. *See, e.g.*, Roth, *Trends Pharmacol. Sci.* 7: 404-407 (1986); Farb and Gibbs, in: *CNS Transmitters and Neuromodulators* CRC Press, New York, NY, pp. 23-36

(1996). However, the complete elucidation of this molecular function requires characterization of the enzyme(s) involved in this process.

Disclosed herein is a novel human cytosolic sulfotransferase that is exclusively-expressed in the nervous system. This novel nervous system-specific sulfotransferase has been designated herein as "SULTn".

The SULTn transcripts were demonstrated to be abundantly expressed in an identified neuronal cell type; the Purkinje cells of the cerebellum and are widely, but discretely localized, within the adult brain. SULTn was also found to be expressed in the developing embryo in all three identified branches of the mammalian nervous system (*i.e.*, the central (CNS), peripheral (PNS), and enteric nervous systems).

II. SULTn Structural Features

A comparison of cytosolic and Golgi-associated sulfotransferases reveals highly-conserved amino acid sequences in the catalytic domain that interact with the co-substrate PAPS. *See, e.g.*, Kakuta, *et al.*, *Nature Struct. Biol.* 4: 904-908 (1997); Kakuta, *et al.*, *TIBS* 23: 129-130 (1998); Weinshilboum, *et al.*, *FASEB J.* 11: 3-14 (1997). It is important to note that these highly-conserved SULTn sequences have not been found in other proteins. *See, e.g.*, Kakuta, *et al.*, *TIBS* 23: 129-130 (1998). The SULTn polypeptide sequence contains homologous PAPS binding motifs, thus suggesting that SULTn is a sulfotransferase. The binding of SULTn to

PAP-agarose indicates that the protein is able to bind PAP.

Further sequence analysis of SULTn provides several lines of evidence that indicate that this polypeptide is related to cytosolic rather than Golgi-associated sulfotransferases. First, SULTn does not have the transmembrane sequence that is found in Golgi-associated sulfotransferases. Second, analysis of sequences that interact with PAPS, establishes that SULTn has sequences in the catalytic regions of the enzyme that are typical of cytosolic sulfotransferases rather than Golgi-associated sulfotransferases. Third, although SULTn does not align with known Golgi-associated sulfotransferases, it does align with cytosolic sulfotransferases. According to the definitions set forth in the guidelines on sulfotransferase nomenclature, SULTn falls below the required 45% amino acid identity threshold value to be a member of known cytosolic sulfotransferases families. *See*, Weinshilboum, *et al.*, *FASEB J.* 11: 3-14 (1997). This result suggests that SULTn defines a new class of cytosolic sulfotransferases. Initially, cytosolic sulfotransferases were named after the specific substrate of the enzyme. Recently, however, it has been suggested that this nomenclature be changed because the cytosolic sulfotransferase enzymes have been found to display broad substrate specificity.

A comparison of the gene structure of human SULTn with human SULT1E (*see*, Her, *et al.*, *Genomics* 53: 284-295 (1995)) and human SULT2A (*see*, Otterness, *et al.*, *DNA*

Cell Biol. 14 331-341 (1995)) revealed that two exons in the center of each sulfotransferase contain an identical number of nucleotides. In addition, the 127 and 95 nucleotide size of these two exons is conserved in guinea pig SULT1E, as well as in rat and human phenol sulfotransferase genes. *See,* Weinshilboum, *et al., FASEB J.* 11: 3-14 (1997). These two exons may be subject to a size constraint as a result of the common function of the sulfotransferase enzymes, such as generating a binding pocket for the co-substrate PAPS.

III. Comparison of SULTn with Other Sulfotransferases Expressed in the Nervous System

SULTn and the Golgi-associated sulfotransferase NSIST (*see, e.g.,* Nastuk, *et al., J. Neurosci.* 18: 7167-7177 (1998)) are both sulfotransferases that are exclusively expressed in the nervous system. However, it is quite likely that these enzymes have different nervous system functions. The Golgi-associated sulfotransferase NSIST, is thought to participate in nervous system-specific post-translational modifications. Conversely, the homology of SULTn to cytosolic sulfotransferases suggests that SULTn substrates are neurochemicals of the cytosol. Although the range of substrates for NSIST has not been elucidated, it is likely to include more than one substrate. *See, e.g.,* Nastuk, *et al., J. Neurosci.* 18: 7167-7177 (1998). Similarly, SULTn may also be important for a range of nervous system cytosolic substrates.

Dopamine sulfotransferase, a phenol sulfotransferase that has a preference for monoamine substrates, has been found to be expressed in the brain, liver, adrenal, testes, gut, and spleen. The gastrointestinal tract has also been shown to be a major site for the production of the majority of circulating dopamine and dopamine sulfate in humans. *See, e.g.*, Eisenhofer, *et al.*, *Clin. Endocrinol. Metabol.* 82 3864-3871 (1997).

The formation of dopamine sulfate has been demonstrated to serve as means of modulating the biological activity of dopamine. In addition, dopamine sulfate functions as a transport intermediate that can be reactivated in target tissues through enzymatic hydrolysis by sulfatases. *See, e.g.*, Yoshizumi, *et al.*, *Brain Res.* 803: 153-160 (1995). As will be discussed *infra*, the restricted expression pattern, which was detected for SULTn by Northern blot and *in situ* hybridization suggested that this polypeptide acts upon a substrate that, in terms of localization of expression, is unlike dopamine. It should be noted that, apart from phenol sulfotransferase, no other known cytosolic sulfotransferases have been detected in the mammalian brain. *See*, Dunn and Klaassen, *Drug Metabol. Disposition* 26: 598-604 (1998).

IV. SULTn Expression Pattern

The Northern blot and *in situ* hybridization patterns of SULTn in the nervous system was found to be consistent with its expression in neurons. SULTn expression was

detected in an identified cell type of the cerebellum and in the ganglion cells of the retina.

Specifically, the astrocytic-specific marker protein, glial fibrillary acidic protein (GFAP), was used to compare the hybridization pattern obtained with SULTn. The expression pattern for SULTn and GFAP was found to be different in the retina, olfactory bulb, and the cerebellum, thus suggesting that SULTn is not expressed in astrocytes. The SULTn hybridization signal in the white matter of the cerebellum appears to be in cerebellar nuclei, although oligodendrocytes may be included in the hybridization signal. Oligodendrocyte-specific markers were to be utilized to determine whether SULTn is expressed in these cells.

V. Specific Examples

A. Database Searches

To search for novel sulfotransferases, the amino acid sequences of known sulfotransferases were used as query sequences in a basic local alignment search tool (BLAST), search of the database of expressed sequence tags (dbEST) available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The following query sequences were used in the search: (*i*) human estrogen sulfotransferase (SULT1E/EST, GenBank Accession No. S77383; *see*, Falany, *et al.*, *J. Steroid Biochem. Mol. Biol.* 52: 529-539 (1995); (*ii*) monoamine-sulfating phenol sulfotransferase (SULT1A3/PST, GenBank Accession No. X84653; Jones, *et al.*, *Biochem. Biophys. Res. Comm.* 208: 855-862 (1995); and (*iii*)

dehydroepiandrosterone sulfotransferase (SULT2A/DHEAST, GenBank Accession No. X70222; Comer, *et al.*, *Biochem. J.* 289: 233-240 (1993).

The tblastn program was used in the dbEST search. The tblastn program compares a protein query sequence against a nucleotide sequence database translated in all reading frames. The sequences retrieved were then examined for novel matches with nervous system expression that gave greater than 30% identity to known sulfotransferases in a region spanning at least 60 amino acid residues. The novel sequences obtained were then used to search dbEST again, to find identical overlapping clones using the blastn program. Thus, the blastn program compares a nucleotide query sequence against a nucleotide sequence database.

It should be noted however, that the scope of the present invention is not to be limited to the specifically-disclosed sequences, but rather encompasses any variation of such sequences which are capable of meeting these two criteria, which possess the ability to: (*i*) hybridize to the disclosed sequences under stringent hybridization conditions; and (*ii*) encode a functional sulfotransferase protein. With respect to the first criteria, an example of stringent hybridization conditions includes, hybridization in which the sequence(s) of interest are fixed to a solid support, and a second nucleic acid molecule to be tested for the ability to hybridize to the disclosed sequences is detectably-labeled and suspended in a hybridization

buffer comprising 50% formamide, 5X SSPE (1 X SSPE is 0.15 mM NaCl, 1 mM Na-EDTA, 10 mM Sodium phosphate (pH 7.0), 5X Denhardt's solution (0.1% polyvinylpyrrolidone, 0.1% Ficoll)). The hybridization buffer is contacted with the solid support at a temperature of approximately 45°C for a period of several hours. The hybridization solution is then removed, and non-specifically bound nucleic acid is removed by repeated washing with, *e.g.*, 1 X SSC at increasing temperatures (*i.e.*, up to 65°C). With respect to the second criteria, the functionality of an encoded product can be determined by a variety of assay techniques known in the art.

B. Isolation of Rat SULTn cDNA

A rat brain cDNA sequence (W74876) was identified as a candidate novel sulfotransferase from the database searches. This cDNA clone was obtained from ATCC (http://www.atcc.org; Manassas, VA) and the entire sequence of this cDNA was determined on both strands using an Applied Biosystems 3773A DNA sequencer (Foster City, CA). The sequence was compiled using the program Sequencher (Gene Codes Corporation, MI). Other computer programs utilized for sequence analysis were obtained from the Wisconsin Package Version 9.1, Genetics Computer Group (http://www.gcg.com; Madison, WI), except where expressly indicated.

The rat SULTn sequence that was obtained has been deposited in GenBank under Accession Number AF176343. The cDNA was cloned uni-directionally into the cDNA cloning site of the pCMV·SPORT2 vector (Life Technologies,

Gaithersburg, MD). The nucleotide and amino acid sequences of the rat SULTn cDNA are shown in SEQ ID NO:1 and SEQ ID NO:2, respectively.

C. Isolation of Human SULTn cDNA

The human SULTn cDNA clone identified in the database searches which comprised the greatest amount of 5' sequence, (GenBank Accession Number AA351518), was obtained from ATCC. Unfortunately, however, none of the cDNA sequences obtained from the bacterial stock matched the associated database entry. Therefore, in order to obtain a human SULTn cDNA clone, two primers (5'-ATGGCGGAGAGCGAGGCCGAGAC-3'; SEQ ID NO:7) and (5'-AGGTCAGAGGGCAGAAAGGGTCAG-3'; SEQ ID NO:8), were used to screen a human fetal brain cDNA library (Genome Systems, St. Louis, MO).

The cDNA clone (19036) obtained from the screening was then inserted in the *Bst*XI and *Not*I sites of the vector pcDNA2.1 (Invitrogen, Carlsbad, CA). The sequence of the human SULTn cDNA was determined on both strands and was subsequently deposited in GenBank under Accession Number AF176342. The nucleotide and amino acid sequences of the SULTn cDNA are shown in SEQ ID NO:3 and SEQ ID NO:4, respectively.

D. SULTn Genomic Sequence

The human SULTn sequence (GenBank Accession No. AF176342) was used to search the database of high-throughput genomic sequences (htgs). A human genomic clone (GenBank Accession No. Z97055;

http//www.sanger.ac.uk/HGP/Chr22) with an identical sequence to the SULTn cDNA was identified and subsequently obtained from the Sanger Center (Cambridge, UK). The nucleotide and amino acid sequences of the genomic SULTn are shown in SEQ ID NO:5 and SEQ ID NO:6, respectively. The genomic DNA was then compared to the human SULTn cDNA sequence to determine the exact intron/exon positions.

E., *In vitro* Translation

The human SULTn cDNA sequence was inserted into pcDNA3.1 as a *Spe*I/*Xba*I fragment. The SULTn pcDNA3.1 construct was linearized with *Xba*I and transcribed with T7 polymerase. A human estrogen sulfotransferase (SULT1E/EST) cDNA clone (GenBank Accession No. AA334071) inserted in the pBluescript vector (Stratagene, La Jolla, CA) was purchased from ATCC (135761) and sequenced to verify its identity. The human SULT1E sequence was then ligated into pcDNA3.1 as an *Eco*RI/*Xho*I-digested insert fragment. The human SULT1E cDNA was linearized with *Not*I and transcribed with T7 polymerase.

Capped RNA was synthesized using message machine (Ambion, Austin, TX). The RNA was subsequently translated *in vitro* using SpeedRead Lysates (Novagen, Milwaukee, WI) in the presence of ^{35}S-methionine. The products of the *in vitro* translation were examined by reducing SDS polyacrylamide gel electrophoresis on a 14% gel (Novex, San Diego, CA). The gel was fixed in isopropanol:water:acetic acid (25:65:10) for 30 minutes, incubated with Amplify™ (Amersham, UK), a fluorographic

reagent, for 15 minutes, dried under vacuum at 80°C and exposed to X-ray film.

F. Binding of SULTn to Adenosine Diphosphate

Following the *in vitro* translation protocol as described *supra*, the reaction products containing the enzyme (25 µl) were mixed with 925 µl of 100 mM Tris-HCl, pH 7.3 and 50 µl of adenosine 3', 5'-diphosphate-agarose (A3640; Sigma, St. Louis, MO) that had been previously washed and equilibrated in 100 mM Tris-HCl, pH 7.3. The enzyme was incubated with the agarose beads for 1 hour at 4°C and then washed a total of 4-times with 1 ml of 100 mM Tris-HCl, pH 7.3, to remove any protein that did not bind to the beads. After the final washing, the sample was heated to 100°C in reducing sample buffer for 5 minutes (Novex, San Diego, CA) and visualized as described above, for the products of the *in vitro* translation.

G. Northern Blot Analysis and *In Situ* Hybridization

Northern blots (Clontech, Palo Alto, CA) were hybridized sequentially to probes for human SULTn and human β-actin (Clontech, Palo Alto, CA). The human SULTn probe corresponded to nucleotides 1-686 of the cDNA sequence (*i.e.*, a *Spe*I/*Pvu*II fragment of the cDNA clone designated pcDNA2.1/19036).

The DNA was subsequently labeled using the random-primed DNA labeling kit (Boehringer Mannheim, Il). Hybridizations were carried out in 6X SSC, 5X Denhardt's, 0.5% (w/v) SDS, 10 mM EDTA, 1 mg/ml denatured salmon

testes DNA, at 65°C. The blots were washed in 2X SSC, 0.1% (w/v) SDS at 23°C for 30 minutes, followed by a wash in 0.2X SSC, 0.1% (w/v) SDS at 60°C for 30 minutes. The blots were then exposed to Kodak BioMax MR film (Rochester, NY) with two intensifying screens at -80°C.

In situ hybridization was performed using ^{35}S-labeled rat SULTn antisense or sense probes. The rat antisense SULTn probe was generated from a *Nco*I digest of the pSPORT2/827634 construct, and corresponded to the 3'-untranslated region comprising nucleotides 1207-1840 of the cDNA sequence (GenBank Accession No. AF176343). The sense probe was synthesized with SP6 and the template was a *Sal*I digest of pSPORT2/827634/*Pst*I, corresponding to nucleotides 1280-1840 of the cDNA sequence (GenBank Accession No. AF176343). The pSPORT2/827634/*Pst*I plasmid construct was generated from the pSPORT2/827634 construct by digesting with *Pst*I, removing the 1.2 kb *Pst*I fragment, and re-ligating the SULTn/SPORT2 vector *Pst*I fragment to generate pSPORT2/827634/*Pst*I. The antisense GFAP probe was synthesized using T7 and a template generated by a *Bam*HI-digest of a 1.27 kb GFAP cDNA in pBK-CMV (Stratagene, La Jolla, CA). *See*, McCall, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93: 6361-6366 (1996).

H. Identification of Sulfotransferase Expressed in the Nervous System

In order to identify novel sulfotransferase cDNA sequences expressed in the brain, BLAST searches of the GenBank database of expressed sequence tags (dbEST) were performed using known sulfotransferase amino acid

sequences as the query (as previously described above in Section A).

A rat brain cDNA (GenBank Accession No. W74876) and a human infant brain cDNA (GenBank Accession No. AA351518) were retrieved. The rat and human cDNA clones contained almost identical sequences encoding a deduced polypeptide with 98% amino acid identity. Therefore, it is assumed that these two cDNAs are clones of orthologous genes. This nervous system-derived sulfotransferase was designated, SULTn.

The SULTn DNA sequence was then used in a BLAST search of dbEST to obtain additional information concerning the expression pattern of SULTn. The human dbEST currently includes entries from brain, heart, kidney, liver, lung, and spleen. *See, e.g.*, Pandey and Lewitter, *TIBS* 24: 276-280 (1999).

A total of 20 human sequences retrieved for SULTn were derived from nervous system or embryonic tissue (*see*, Table 1) indicating that the expression pattern of this novel sulfotransferase fit the search criteria which was established for a nervous system sulfotransferase. Moreover, a total of six of the database entries were derived from multiple sclerosis lesions or from brains of muscular atrophy patients, thus raising the possibility that SULTn may be up-regulated in these aforementioned disease states. Table 1 illustrates the expressed sequence tag (EST) sequences retrieved with a BLAST search. The table reveals that the expression of the SULTn cDNA is specific to the nervous system. The

GenBank accession numbers (accession) for the 21 human EST sequences for the SULTn sulfotransferase are tabulated along with the tissue (tissue) source, where known, the sequence submitter (submitter) and the length of the EST sequence in base pairs (bp). The stages of the tissue preparation are indicated in days (d), weeks (wk), months (m) and years (yr).

Interestingly, SULTn had no liver database entry in dbEST. This finding is in direct contrast to various other sulfotransferases (*e.g.*, estrogen sulfotransferase, phenol sulfotransferase, and the like), for which the majority of database entries are derived from liver tissue. Liver has been shown by Northern blot analysis to express each of seven cytosolic sulfotransferase enzymes previously identified (*see, e.g.*, Dunn and Klaassen, *Drug Metabol. Disposition* 26: 598-604 (1998)), thus highlighting the unusual lack of expression of SULTn in the liver as found by the database searches.

One additional rat SULTn entry was obtained (GenBank Accession No. H31054) from the database search using the SULTn DNA sequence as the query. The rat cDNA was isolated from a comparative study of sequences expressed in the pheochromocytoma cell line PC12, in response to the administration of nerve growth factor. *See,* Lee, *et al., Proc. Natl. Acad. Sci. U.S.A.* 92: 8303-8307 (1995). PC12 cells have been demonstrated to possess many of the characteristics of adrenal chromaffin cells, but differentiate into cells with sympathetic neuron

characteristics in response to administration of nerve growth factor.

I. Human SULTn and Potential 3'-Phosphoadenosine 5'-Phosphosulfate Binding Sites

A clone (GenBank Accession No. AF176342) comprising the complete coding sequence of SULTn was obtained by screening a human fetal brain cDNA library (as described in Section B, *supra*). The nucleotide (SEQ ID NO:3) and deduced amino acid (SEQ ID NO:4) sequences of human SULTn are illustrated in FIG. 1. The cDNA sequence was found to contain 2419 nucleotides and encodes a predicted 284 amino acid residue polypeptide. A polyadenylation signal (AATAAA) is located 19 nucleotides 5' of the poly(A) signal. The partial SULTn sequence which was obtained by the sequencing of the rat cDNA clone, was found to comprise an insert of 1840 base pairs encoding an open reading frame (ORF) of 160 amino acid residues (GenBank Accession No. AF176343). The start of the aforementioned rat sequence is indicated by an arrow on the human SULTn in FIG. 1.

An alignment of human SULTn with the 160 carboxyl-terminal amino acid residues of the rat SULTn sequence revealed a total of only three different amino acids - with serine(189), alanine(225), and threonine(227) in human SULTn being substituted with alanine, serine and isoleucine in rat SULTn, respectively. These results indicate that the SULTn protein sequence is highly conserved in different species.

Both cytosolic and Golgi-associated sulfotransferases use 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as a co-substrate. *See*, Kakuta, *et al.*, *TIBS* 23: 129-130 (1998). Consensus sequences that are involved in PAPS binding in both cytosolic and Golgi-associated sulfotransferases have been proposed from sequence alignments, mutational analysis, and interpretation of the estrogen sulfotransferase crystal structure. *See, e.g.*, Kakuta, *et al.*, *Nature Struct. Biol.* 4: 904-908 (1997); Kakuta, *et al.*, *TIBS* 23: 129-130 (1998); Weinshilboum, *et al.*, *FASEB J.* 11: 3-14 (1997); Berninsone and Hirschberg, *J. Biol. Chem.* 273: 25556-25559 (1998); Ong, *et al.*, *J. Biol. Chem.* 274: 25608-25612 (1999). Three conserved sequences have been implicated in the interaction of sulfotransferases with the 5'-phosphosulfate and the 3'-phosphate of PAPS. Although these sequences are conserved in sulfotransferases, they have not been identified in other proteins. *See, e.g.*, Kakuta, *et al.*, *TIBS* 23: 129-130 (1998).

The alignment of human SULTn with three motifs that are conserved in sulfotransferase are also illustrated in FIG. 1. The 5'-phosphosulfate binding consensus sequence in cytosolic and Golgi-associated sulfotransferases has been shown to comprise the amino acid sequence PK(T/S)GTT(A/W)L. *See*, Kakuta, *et al.*, *TIBS* 23: 129-130 (1998). As illustrated in FIG. 1, Panel B, SULTn has a total of six out of eight identical matches in the 5'-phosphosulfate binding motif. This motif is preceded by the amino acid sequence TY in SULTn (*see*, FIG. 1, Panel

A), a feature that is typical of cytosolic, but not Golgi-associated sulfotransferases.

The first, 3'-phosphate binding sites found in cytosolic and Golgi-associated sulfotransferases are illustrated in FIG. 1, Panel C. *See, e.g.*, Kakuta, *et al.*, *TIBS* 23: 129-130 (1998). As illustrated in FIG. 1, Panel D, the second 3'-phosphate binding site has only been described in cytosolic sulfotransferases. *See, e.g.*, Weinshilboum, *et al.*, *FASEB J.* 11: 13-14 (1997). As illustrated in FIG. 1, SULTn has a total of 11 out of 16 amino acid residues identical and 10 out of 13 amino acid residues identical, in the first and second 3'-phosphate binding motifs, respectively.

The partial rat SULTn sequence includes the two PAPS 3'-phosphate binding sites, and these sequences are identical to the human SULTn sequence. Apart from the PAP binding sites, no other region(s) of identity was detected between SULTn and Golgi-associated sulfotransferases. Collectively, analysis of the potential PAPS binding sites and the lack of a hydrophobic region of amino acid residues that might act as a transmembrane sequence, suggest that SULTn is a cytosolic rather than a Golgi-associated sulfotransferase.

Table 2 illustrates in the percent identity and percent similarity of human SULTn with other human cytosolic sulfotransferase enzymes. The percentage of amino acid residue identity and similarity between the nervous system-expressed sulfotransferase (SULTn) and:

(*i*) human estrogen sulfotransferase (SULT1E/EST, GenBank Accession No. Y11195); (*ii*) monoamine-sulfating phenol sulfotransferase (SULT1A3/PST, GenBank Accession No. X84653); and (*iii*) dehydroepiandrosterone sulfotransferase (SULT2A/DHEAST, GenBank Accession No. X70222) was calculated using the Genetics Computer Group program " Bestfit" . The percentage identities are shown in Column A and the similarities, taking conservative changes into consideration, are shown in Column B.

As illustrated in Table 2, SULT1E/EST (*see, e.g.*, Falany, *et al.*, *J. Steroid Biochem. Mol. Biol.* 52: 529-539 (1995)) showed 39% identity to monoamine-sulfating phenol sulfotransferase (SULT1A3/PST; *see, e.g.*, Jones, *et al.*, *Biochem. Biophys. Res. Comm.* 208: 855-862 (1995)) and 34% identity to dehydroepiandrosterone sulfotransferase (SULT2A/DHEAST; *see, e.g.*, Comer, *et al.*, *Biochem. J.* 289: (1993); Table 2). The alignment of these amino acid sequences (*see*, FIG. 2) confirms that SULTn is a member of the cytosolic sulfotransferase gene family. As illustrated in FIG. 2, one sequence (YGSW) is invariant between the sulfotransferase sequences, but the function of this sequence is not known. The most closely-related of the cytosolic sulfotransferases shown in Table 2, are SULT1E and SULT1A3 (*i.e.*, exhibiting 49% identity and 59% similarity) which are members of the same sub-family. It should be noted that SULTn shares approximately the same percentage similarity and identity to each of the sulfotransferases set forth in Table 2.

J. Human SULTn Chromosomal Localization

The human SULTn cDNA sequence was used to search the database of high-throughput genomic sequences (htgs), generated as part of the Human Genome Project. A PAC genomic clone that comprised an exact match with human SULTn was identified (Genbank Accession No. Z97055). A group at The Sanger Center, mapping chromosome 22, has localized this PAC clone to chromosome 22. The PAC clone (388MS), containing a total of 177,568 basepairs, was isolated from a chromosome 22 clone contig. No other sulfotransferase genes are located within this clone. Two PAC clones (Genbank Accession No. AL023654 and No. AL023801) with overlapping sequence to the SULTn genomic clone, were mapped to chromosome 22 position 22q13. The schizophrenia-susceptibility locus (600850, SCZD4; *see, e.g.*, Coon, *et al.*, *Am. Med. Genet.* 54: 59-71 (1994)) and cerebellar ataxia (603516, SCA10; *see, e.g.*, Zu, *et al.*, *Am. J. Hum. Genet.* 64: 594-599 (1999) also have been mapped to this chromosomal region. Moreover, the chromosomal localization of SULTn is also distinct from the four chromosomal locations for other cytosolic sulfotransferase genes in human. *See, e.g.*, Her, *et al.*, *Genomics* 53: 284-295 (1998).

Within the human genome: (*i*) three phenol sulfotransferase genes are clustered at 16p11.2; (*ii*) SULT1C1 and SULT1C2 are localized at 2q11.1-q11.2; (*iii*) SULT2A and SULT2B are localized at 19q13.3; and (*iv*) SULT1E is localized at 4q13.1. Similarly, a human tyroprotein sulfotransferase (a Golgi-associated enzyme) has been localized to chromosome 22 at position 22q12.1

(*see, e.g.*, Beisswanger, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 95: 1134-1139 (1998)), which is in proximity to the SULTn gene.

K. Human SULTn Genomic Structure

The location of intron/exon boundaries in the SULTn gene (*see,* Table 3) was determined by aligning the human SULTn cDNA and genomic sequence of PAC clone 388MS. Table 3 illustrates the intron and exon junctions in the SULTn gene. The novel human sulfotransferase (SULTn) was shown to be encoded by a gene comprising a total of 7 coding exons, spanning 47,871 basepairs. The exon sequences at the splice junctions are shown in capital letters and the intron sequences are shown in lower case. The conserved sequences at the splice junctions are shaded in gray. The intron and exon sizes are given in basepairs (bp). The U2-Type GT-AG intron consensus sequence is shown below the SULTn intron/exon junctions.

This analysis revealed that the human SULTn gene contains more than twice the size of the human SULT1E and SULT2A genes, which span approximately 19 and 15.5 kilobase pairs, respectively. *See, e.g.*, Her, *et al.*, *Genomics* 29: 16-23 (1995); Otterness, *et al.*, *DNA Cell Biol.* 14: 331-341 (1995).

These three phenol sulfotransferase genes have been shown to span a region of 4.4-7.3 kilobases of genomic DNA. *See, e.g.*, Weinshilboum, *et al.*, *FASEB J.* 11: 13-14 (1997). The first SULTn intron comprises 20,281 basepairs, which accounts for the majority of the larger size of the SULTn gene. The SULTn intron sequences each

match U2-type GT-AG introns (*see, e.g.*, Sharp and Burge, *Cell* 91: 875-879 (1997); Table 3), rather than the three rarer types of introns. Additionally, human SULT1E, SULT1A3, and SULT2A have also been shown to possess U2-type GT-AG introns.

L. Conserved Exons in the Cytosolic Sulfotransferase Gene Family

FIG. 3 illustrates a comparison of the gene structure of human SULTn with human SULT1E (*see, e.g.*, Her, *et al.*, *Genomics* 29: 16-23 (1995)) and human SULT2A (*see, e.g.*, Otterness, *et al.*, *DNA Cell Biol.* 14: 331-341 (1995)). Two exons in the center of each sulfotransferase contain an identical number of nucleotides. The location of the amino acids encoded by the identically-sized, 127 and 95 nucleotide exons is illustrated in FIG. 2. Interestingly, however, the number and size of other exons of the SULTn gene is not conserved among the various sulfotransferases.

M. Characterization of Recombinant SULTn Polypeptides

The human SULTn cDNA was transcribed *in vitro* and subsequently translated in a rabbit reticulocyte lysate in the presence of ^{35}S-methionine. The translation products were then resolved on a reducing SDS polyacrylamide gel. The following were added to the *in vitro* translation system: no RNA, β-galactosidase RNA, SULT1E RNA, and SULTn RNA. The electrophoretic results demonstrated that no translation products were found when no RNA was added to the rabbit reticulocyte lysate,

whereas the *in vitro* translations products of β-galactosidase and SULT1E were found to migrate at the expected sizes of 119,000 Mr and 35,000 Mr, respectively. During electrophoresis, a polypeptide with an apparent Mr of 33,000 was observed in the lane corresponding to the translated products of the SULTn RNA. This was the predicted size of the SULTn polypeptide based on the conceptual translation of the cDNA sequence. *See*, FIG. 1.

N. SULTn Binds to PAP-Agarose

PAP has been shown to be a potent dead-end inhibitor of sulfotransferases, having an affinity for the enzyme in approximately the same range as that of the sulfonate donor, PAPS. *See, e.g.*, Roth, *Trends Pharmacol. Sci.* 7: 404-407 (1986). Accordingly, the binding of SULTn to PAP-agarose was tested to determine if the PAPS-binding sites detected in the SULTn sequence function in the binding of the PAP analogue of the co-substrate. SULTn binds to PAP-agarose. SULT1E, and β-galactosidase, served as positive and negative controls in the same experiment, respectively.

A competition experiment was then performed by the addition of PAP and PAP-agarose to SULT1E and SULTn proteins. The addition of 60 μM PAP was shown to decrease the binding of SULT1E and SULTn to PAP-agarose, thus demonstrating the specificity of the observed binding to PAP-agarose. These results indicated that the

consensus PAP sequences found in SULTn, function in PAP binding.

O. Expression of Human SULTn

The expression of the human SULTn gene in normal human tissues was examined by Northern blot hybridization analysis of polyA+ RNA. The probe used to detect SULTn transcripts in the hybridization corresponded to the region encoding the amino-terminus. A specific hybridizing species of approximately 2.4 kilobases was detected in human brain mRNA for human SULTn, but not in heart, placenta, lung, liver, skeletal muscle, kidney, and pancreas. As previously discussed, the human SULTn cDNA clone (SEQ ID NO:3) possesses an insert comprising 2419 basepairs. This insert size shows excellent agreement with the size of the SULTn transcripts which were detected.

More specifically, SULTn transcripts were detected in the human cerebral cortex, frontal lobe, occipital pole, temporal lobe, cerebellum, whereas no signal was detected for SULTn in either the spinal cord or medulla. The results of these Northern blots confirm the findings from the previously-discussed expressed sequence tag (EST) database searches that SULTn is expressed in the nervous system and not in the liver, unlike other previously-described cytosolic sulfotransferases.

P. *In situ* Hybridization of SULTn in the Rat CNS

The cellular localization of SULTn transcripts in the rat nervous system were examined by *in situ*

hybridization using a rat SULTn-specific probe directed against the 3'-untranslated region. SULTn transcripts were detected in the accessory, granular, and glomerular cell layer of the olfactory bulb.

Glial fibrillary acidic protein (GFAP), an astrocytic-specific marker, was used to hybridize adjacent olfactory bulb sections, for comparison with the SULTn hybridization signal. GFAP was detected in the glomerular layer of the olfactory bulb, although the hybridization pattern for SULTn and GFAP were found to differ. Additionally, an adjacent olfactory bulb section was hybridized with the SULTn sense probe and no hybridization signal was detected. SULTn expression was also detected in the hippocampus and the cortex.

In the cerebellum, SULTn transcripts were detected in Purkinje cells and in cerebellar nuclei, located in the trunk of the cerebellar white matte. However, these hybridization signals were not observed with the SULTn sense probe. Interestingly, no hybridization signal was observed for GFAP in the region of the cerebellar nuclei, whereas a hybridization signal was detected for GFAP in the molecular layer of the cerebellum, corresponding to basket and stellate cells.

The retina was examined for SULTn expression and hybridization signals were detected in the retinal ganglion cell layer. GFAP was detected in the retinal ganglia cell layer, but the pattern of hybridization was distinct.

Q. *In situ* Hybridization of SULTn in the Embryo

The hybridization pattern of SULTn in the developing embryo was examined by *in situ* hybridization. Embryos of embryonic day 11, 14 and 17 (designated E11, E14, and E17, respectively) were hybridized with SULTn. The first developmental stage that exhibited SULTn expression was found to be the day 11 E11 embryo. The transcripts that were detected were localized in the developing cranial ganglia.

In the day 14 E14 embryo, hybridization was detected in the developing central, peripheral, and enteric nervous system. Specifically, SULTn transcripts were detected in the developing cortex, midbrain, trigeminal ganglia, spinal cord, dorsal root ganglia, and enteric ganglia. No signal was detected outside the nervous system.

The SULTn hybridization pattern in the E17 embryo was similar to that obtained with the E14 embryo. Specifically, hybridization results for the E17 embryo using the SULTn antisense probe demonstrated expression of SULTn in the cortex, hippocampus, basal ganglia, midbrain, thalamus, and cranial ganglia. Similar results were also found for E17 using SULTn sense probe hybridization. These aforementioned results indicate that embryonic expression of SULTn is restricted to the nervous system.

It should be noted that the present invention is not to be limited in scope by the specific embodiments described herein. Moreover, various modifications of the

present invention in addition to those described herein will become apparent to those skilled in the art from the foregoing descriptions and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Table 1

Human Tissue	DbEST Accession Number
Embryo (6 wk)	AA330927
Brain, fetal	AA340331
Brain, infant	AA351518
Brain, muscular atrophy patient female (3m)	F10349
Brain, muscular atrophy patient female (3m)	F12742
Brain, post natal female (73 d)	H10341
Brain, post natal female (73 d)	H10342
Brain, male (55 yr)	H20428
Brain, male (55 yr)	H20429
Retina	L48891
Multiple sclerosis lesions, male (46 yr)	N63574
Multiple sclerosis lesions, male (46 yr)	N94830
Brain, post natal female (73 d)	R38615
Brain, muscular atrophy patient female (72 d)	T15470
Brain, post natal (73 d)	T33208
	T75156
Retina, adult	W25958
Retina, adult	W27307
Retina, adult	W26950
Brain, infant muscular atrophy patient	Z38771
Brain, female (3m)	Z42591

Table 2

		EST	PST	DHEAST
A	Identities			
	NST	36	39	34
	EST		49	37
	PST			36
B	Similarities			
	NST	45	46	43
	EST		59	47
	PST			46

Exon	Exon Size(bp)	3'-Exon Junction	Splicing Donor	Intron Size(bp)	Splicing Acceptor	5'-Exon Junction	Exon
1	169	-CCCAAGTCCG	gagccccg-	1:20281	-tcccctcg	GCACCAGCTT-	1
2	131	-CATCATCAAG	cccaggg-	2: 1775	-ctccacgg	GAACTGACCT-	2
3	81	-AGACTCCAAG	agctggc-	3: 951	-tcacccac	GTCATCTATA-	3
4	127	-AATGATAAGC	ggttg-	4: 5132	-gtctccgc	TGGGCTACGG-	4
5	95	-CATGCATCGG	gctg-	5: 4440	-tgtcttcc	ACCTGGTGA-	5
6	139	-GTGGGCCCGG	gccc-	6:12946	-tgcgtttc	AAGAGTTGG-	6
7	1604						
U2-Type GT-AG Intron consensus							

Table 3

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a nervous system-specific sulfotransferase, wherein said nucleic acid sequence is selected from the group of sequences consisting of: SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

2. An isolated polypeptide comprising a nervous system-specific sulfotransferase, wherein the amino acid sequence of said polypeptide is selected from the group of sequences consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6.

3. An antibody capable of immunospecifically-binding to a polypeptide comprising a nervous system-specific sulfotransferase, wherein the amino acid sequence of said polypeptide is selected from the group of sequences consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6.

4. An isolated nucleic acid comprising a first nucleic acid sequence encoding a nervous system-specific sulfotransferase which has the ability to hybridize to a second nucleic acid sequence under stringent hybridization conditions, wherein said second nucleic acid sequence is selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

5. A method for identifying an effector of nervous system-specific sulfonation, said method comprising:

a) providing a nervous system-specific sulfotransferase polypeptide encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5;

b) contacting said polypeptide of step a) with a candidate effector compound under conditions appropriate for the binding of said candidate inhibitor compound to the polypeptide of step a); and

c) assaying for the ability of said candidate effector compound of step b) to effect the sulfotransferase function of the polypeptide of step a).

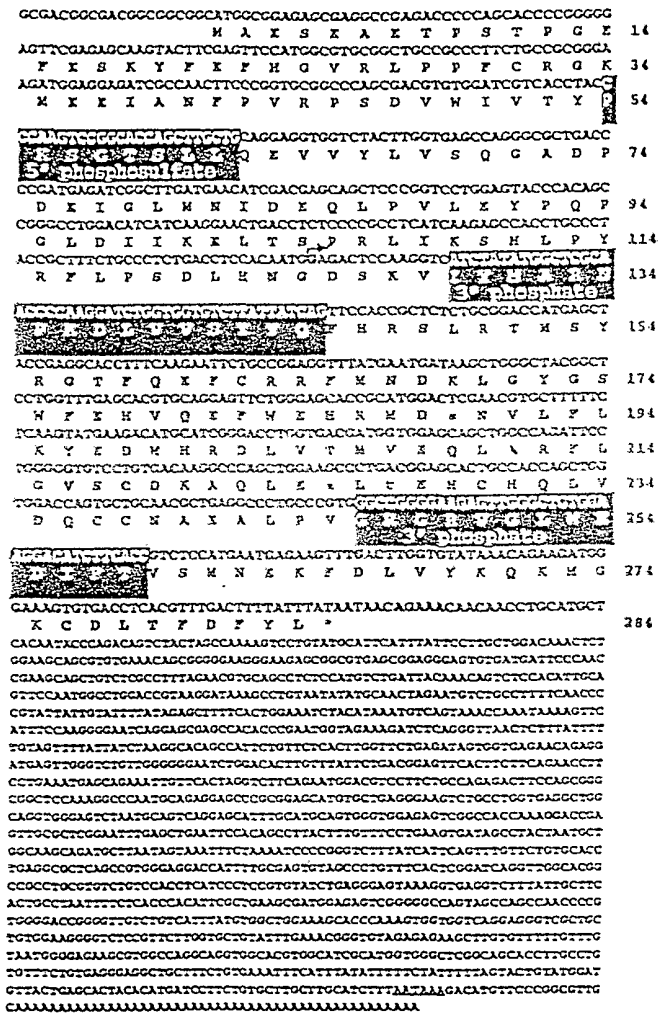
6. A method for inhibiting nervous system-specific sulfonation, said method comprising contacting, under physiological conditions, a polypeptide encoded by the nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, , SEQ ID NO:3, and SEQ ID NO:5, with a compound capable of interacting with and inhibiting the sulfonation activity of said encoded polypeptide.

7. The method of Claim 6, wherein said compound is selected from the group consisting of: an antibody, a peptide, a polypeptide, a nucleic acid, an organic molecule, and an inorganic molecule.

8. A method of treating a neurological disorder, said method comprising administering a biologically-effective amount of a nervous system-specific sulfotransferase polypeptide encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5, wherein said polypeptide is an effector of nervous system-specific sulfonation.

9. The method of Claim 8, wherein said neurological disorder is selected from the group consisting of: multiple sclerosis, nervous system-mediated muscular atrophy, amyotrophic lateral sclerosis, Huntington's chorea, myasthenia gravis, Alzheimer's Disease, senile dementia, schizophrenia, bipolar disorder, and cerebellar ataxia.

A



B

PAPS 5' phosphosulfate binding site



C

PAPS 3' phosphate binding site



D

PAPS 3' phosphate binding site



FIG 1

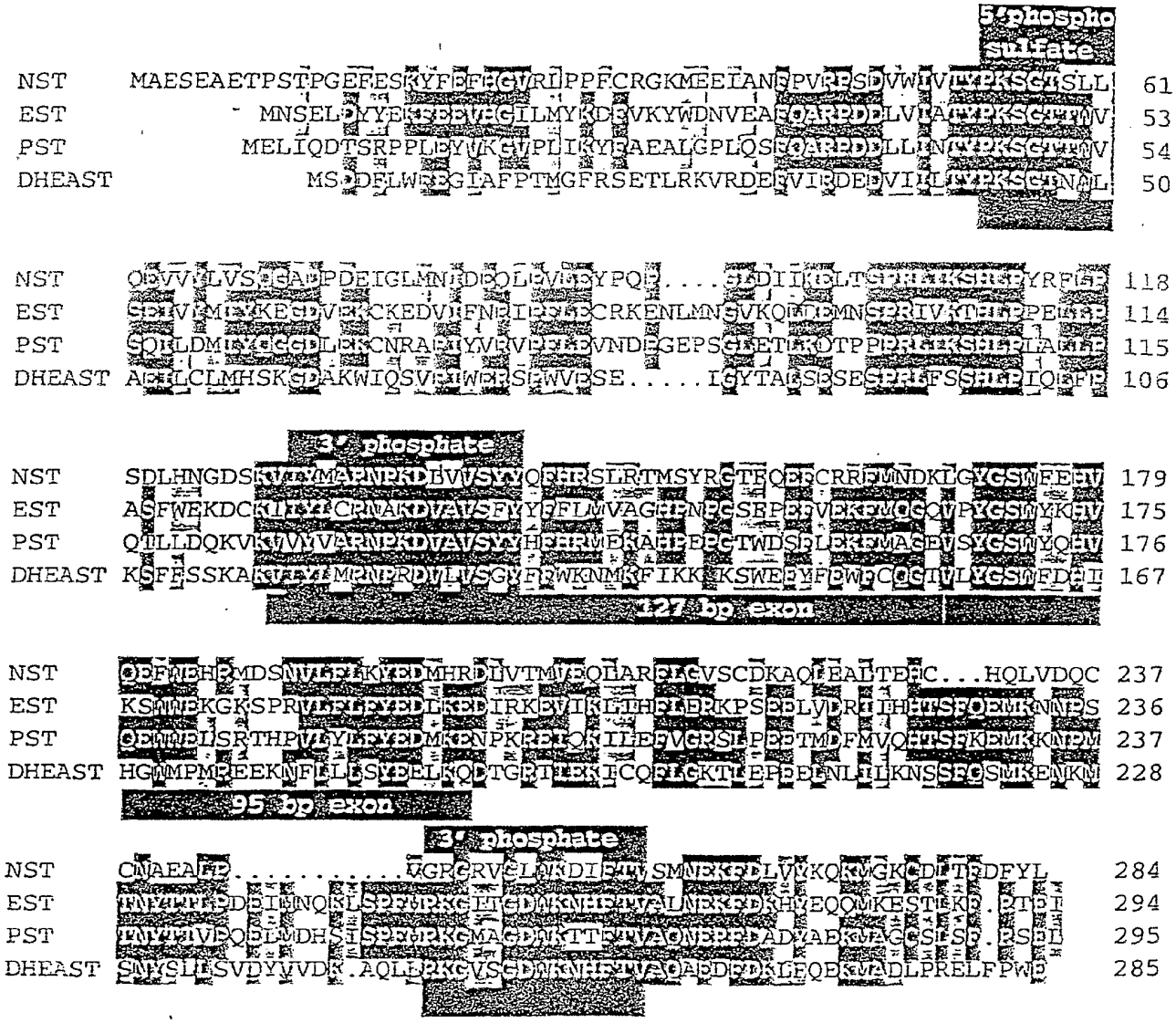

5' phospho sulfate
NST
EST
PST
DHEAST
3' phosphate
127 bp exon
95 bp exon
3' phosphate

FIG 2

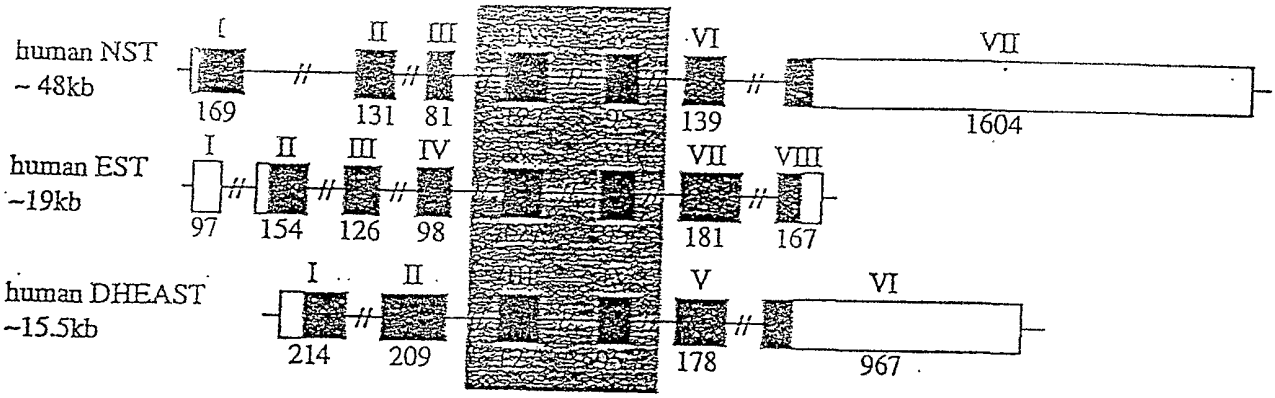

human NST
~ 48kb
I
II
III
VI
VII
169
131
81
139
1604
human EST
~19kb
I
II
III
IV
VII
VIII
97
154
126
98
181
167
human DHEAST
~15.5kb
I
II
V
VI
214
209
178
967

FIG 3