Molecular cloning and expression of novel sulphotransferase-like cDNAs from human and rat brain

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The sulphotransferase (SULT) gene family is involved with the conjugation of many small drugs, xenobiotics and endogenous compounds. In this report, we describe the cloning and expression of novel cDNAs from human and rat brain, which are structurally related to the SULTs. These cDNAs have been termed 'brain sulphotransferase-like' (BR-STL), because of their similarity to the SULTs and their selective expression in brain tissue. The proteins encoded by the human and rat BR-STL cDNAs (hBR-STL-1 and rBR-STL cDNA respectively), denoted as hBR-STL and rBR-STL, are 98% identical and 99% similar in sequence. The hBR-STL-1 cDNA contains an 852-nt open reading frame encoding a 284-amino-acid protein with a calculated molecular mass of 33083 Da. Northern-blot analyses of RNA isolated from eight human tissues indicate that the hBR-STL message is selectively expressed in brain. Characterization of different brain regions showed that message levels were highest in cortical brain regions. rBR-STL message levels were relatively low in brains of 1-day-old male and female rats, but increased to adult levels in RNA from 7-day-old rats, and remained at that level in adult animals. The hBR-STL and rBR-STL cDNAs were expressed in both *Escherichia coli* and Sf9 insect cells in the presence or absence of an N-terminal histidine-affinity tag (His-tag). Polyclonal antibodies were raised in chickens against purified His-tagged hBR-STL, and were used to detect the presence of rBR-STL in adult male and female rat brain cytosol. The high degree of sequence conservation, and the selective localization of the BR-STL message in brain, suggest an important function in the central nervous system.

Key words: central nervous system, Phase II conjugation, rat.

INTRODUCTION

Sulphation is an important Phase II or conjugation reaction involved in the metabolism of drugs, xenobiotics and endogenous compounds [1]. Conjugation of a compound with a charged sulphonate moiety usually results in a decrease in the biological activity, and an increase in the aqueous solubility and excretion, of the compound. Sulphate conjugation of endogenous chemicals, such as thyroid hormones, steroids and monoamine neurotransmitters, is important in the biosynthesis, metabolism and modulation of the biological activity of these compounds [1].

A superfamily of enzymes known as the sulphotransferases (SULTs) are responsible for the sulphation reaction. The SULT superfamily consists of two families: one family consists of the membrane-associated SULTs involved in the sulphation of glycosaminoglycans, glycoproteins and tyrosines in peptides and proteins [2]; the second is the cytosolic family of SULTs, which are responsible for the conjugation of xenobiotics, drugs and small endogenous compounds, such as steroids and monoamine neurotransmitters. At least nine distinct isoforms of cytosolic SULT have been identified in human tissues. The SULT isoforms can be grouped into two families: (i) the phenol SULTs [P-PST-1 (SULT1A1), P-PST-2 (SULT1A2), M-PST (SULT1A3), EST (SULT1E1), ST1B2 (SULT1B2), ST1C1 (SULT1C1) and ST1C2 (SULT1C2)], and (ii) the hydroxysteroid SULTs [DHEA-ST (SULT2A1) and ST2B1a and ST2B1b (SULT2B1a and SULT2B1b)]. Although the SULT genes are most highly expressed in liver, the cytosolic SULTs are present in many extrahepatic tissues, including the small intestine, adrenal gland, kidney, skin, brain, blood platelets and nucleated blood cells. Several of the SULTs have been identified in human brain tissues [3–5], and the phenol SULTs have been immunolocalized in neurons in several different brain regions [6].

In the present report, we describe the isolation and expression of a novel SULT-like (STL) cDNA from both human and rat brain cDNA libraries. These STL cDNA sequences were highly conserved at both the nucleotide and amino-acid levels. Comparison of the translated sequences of these cDNAs indicates that they are members of the SULT gene family. Analysis of the expression of the message for these cDNAs indicates that they are selectively expressed in brain tissue. Localization of these SULT-like proteins in brain tissue suggests that they may have a role in the sulphation of drugs and neurotransmitters in the central nervous system.

EXPERIMENTAL

Materials

Taq polymerase was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Restriction enzymes were obtained from New England Biolabs (Beverly, MA, U.S.A.) or Promega (Madison, WI, U.S.A.). The TA-cloning kit was purchased from Invitrogen (San Diego, CA, U.S.A.). Elongase, the BAC-to-BAC Baculovirus expression system, and the SuperScript™ human brain cDNA library were obtained from Life Technologies (Gaithersburg, MD, U.S.A.). STAT-60 was from Tel-Test, Inc. (Friendswood, TX, U.S.A.) The λUni-Zap XR rat

Abbreviations used: SULT, sulphotransferase; BR-STL, brain sulphotransferase-like; hBR-STL-1, human BR-STL; rBR-STL, rat BR-STL; pfu, plaque-forming units; poly(A)$^{+}$, polyadenylated; LB, Luria–Bertani; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; RT, reverse transcriptase.

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The sequences for the human and rat BR-STL cDNAs have been assigned the GenBank accession numbers AF188698 and AF188699 respectively.

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brain cDNA library was obtained from Stratagene (La Jolla, CA, U.S.A.). [^{32}P]dCTP (3000 Ci/mmol) was purchased from DuPont/NEN (Boston, MA, U.S.A.). Oligonucleotide primers were synthesized in the Molecular Biology Core Facility of the Comprehensive Cancer Center (University of Alabama at Birmingham). The Luminol chemiluminescence substrate kit was obtained from Pierce Chemical Co. (Rockford, IL, U.S.A.). Frozen Sprague–Dawley rat brains were purchased from Harlan Bioproducts for Science (Indianapolis, IN, U.S.A.). Adult Sprague–Dawley rats were obtained from Charles River Co. (Wilmington, MA, U.S.A.). All other chemicals were of reagent grade quality.

Isolation of human and rat brain STL cDNAs (hBR-STL-1 and rBR-STL cDNAs respectively)

Initially, a SULT-related partial cDNA fragment was isolated from human pancreatic-islet RNA during an investigation of this tissue using differential display to identify transcripts enriched in human islet cells relative to pancreatic exocrine tissue [7]. A 312-bp DNA fragment was isolated during this process, and subcloned into pBluescript SK$^+$. The fragment was ^{32}P-labelled by the random priming technique using an Oligolabelling kit (Pharmacia, Piscataway, NJ, U.S.A.) to a specific radioactivity $> 1 \times 10^{-9}$ c.p.m./μg, and was used as a hybridization probe to screen 5×10^5 plaque-forming units (pfu) from a human insulinoma cDNA library cloned into λZAP II [8]. Hybridization was performed in 50 % (v/v) formamide, 80 mM Pipes, 2 % (w/v) SDS and 100 mg/ml denatured salmon sperm DNA at 42 °C for 16–20 h. Filters were washed in 0.5 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate) with 0.1 % (w/v) SDS at room temperature, then at 60 °C, and exposed to autoradiography film with an intensifying screen for approx. 48 h. Several plaques that showed hybridization signals on replica filters were purified by dilution and re-screening. The pBluescript sequence containing the unique sequence was excised from λZAP II for sequencing using an ABI 373A automated sequencer. Sequences were compared with the non-redundant nucleic acid and protein databases using BLASTN and BLASTX algorithms [9]. Two partial SULT-related cDNAs were isolated and sequenced.

No hybridization signal was obtained upon Northern-blot analysis of total RNA from human islet tissue or polyadenylated [poly(A)$^+$] RNA from total pancreas, suggesting that only low levels of BR-STL are transcribed in pancreatic endocrine cells. Because screening of a human islet-cell tumour library resulted in the isolation of cDNAs containing intronic sequences, it is plausible that these do not express functional full-length proteins. Full-length human STL cDNAs were isolated from a human brain SuperScript pCMV-Sport plasmid cDNA library using a partial pancreatic BR-STL cDNA as a probe. To screen the library, approx. 15000 bacteria were spread on 150-mm Luria–Bertani (LB) broth/ampicillin plates, and grown overnight at 30 °C. The bacteria were lifted on to nitrocellulose filters, denatured, neutralized, and plasmid DNA was then covalently bound to the filters using a Bio-Rad Gene Linker apparatus. The filters were probed with [^{32}P]dCTP (3000 Ci/mM) pancreatic STL cDNA at a concentration of 500000 c.p.m./ml, and were incubated overnight at 65 °C. The filters were then washed at 65 °C twice with 2 × SSC/0.1 % (w/v) SDS for 15 min, and once with 2 × SSC for 15 min. The filters were then dried and exposed to autoradiography film with an intensifying screen overnight at −70 °C. Colonies that were positive on replicate filters were purified by repeated cycles of dilution and re-screening until single colonies could be isolated. This procedure resulted in the isolation of three pure bacterial clones from the screening of approx. 100000 bacteria of the cDNA library.

The rBR-STL cDNA was isolated from a rat brain λUni-ZAP XR cDNA library by a phage library screening procedure, using the hBR-STL cDNA as a probe [10]. *Escherichia coli* XL-1 Blue MRF′ cells were grown overnight at 30 °C in LB broth supplemented with 0.2 % (v/v) maltose and 10 mM $MgSO_4$. The cells were infected with aliquots of the rat brain cDNA library, plated at approx. 30000 pfu/150-mm Petri plate, and incubated overnight at 37 °C. The phage were transferred to nitrocellulose filters and screened with ^{32}P-labelled hBR-STL cDNA, essentially as described above. The cDNAs were recovered in pBluescript plasmids using ExAssist helper phage following the manufacturer's instructions (Stratagene).

The STL cDNAs were sequenced using a Thermo Sequenase cycle sequencing kit and adenosine 5′-[α-[^{35}S]thio]triphosphate to label the newly synthesized strands. The ^{35}S-labelled products were resolved on 6 % polyacrylamide/urea gels using a buffer gradient of 0.5 × to 2.5 × Tris/borate/EDTA (TBE; where 1 × TBE is 45 mM Tris/borate/1 mM EDTA). The complete cDNA sequences were obtained by subcloning restriction fragments into pBluescript for sequencing with T3 and T7 primers, or by sequencing short fragments generated by *Sau*3A or *Hae*III digestion and subcloned into pBluescript. Synthetic oligonucleotide primers synthesized to internal sequences of the cDNAs were also used to sequence specific regions. Sequence gels were read manually and analysed using MacVector sequence-analysis software.

Baculovirus expression of hBR-STL and rBR-STL cDNAs

The hBR-STL and rBR-STL cDNAs were expressed in insect cells using the BAC-to-BAC Baculovirus Expression System. hBR-STL cDNA was expressed in either the presence or absence of an N-terminal histidine-affinity tag using the pFASTBAC1 and pFASTBACHTB vectors respectively. rBR-STL was expressed as native protein using the pFASTBAC1 vector.

To insert the hBR-STL cDNA into pFASTBAC1 or pFASTBACHTB, PCR was used to generate a *Bam*HI restriction enzyme site immediately upstream of the initial ATG codon of the cDNA to facilitate subcloning. The sense primer (5′-GCG-GATCCATGGCGGAGAGCGA-3′) contained the *Bam*HI site (the initial ATG codon is underlined). The antisense primer corresponded to nt 2179–2157 of the hBR-STL cDNA, and the position of the primer was 3′ of a unique *Hin*dIII restriction site (2020–2025) in the 3′ non-translated region of the hBR-STL cDNA. The hBR-STL cDNA was amplified using Elongase, digested with *Bam*HI and *Hin*dIII, isolated from an agarose gel after electrophoresis by using a QIAquick Gel Extraction kit, and then ligated into pFASTBAC1, which was digested beforehand with *Bam*HI and *Hin*dIII. *E. coli* DH5α competent cells were transformed with the pFASTBAC1-hBR-STL plasmid, and selected for ampicillin resistance. Plasmid DNA was prepared from individual colonies, and the different pFASTBAC-hBR-STL plasmids were confirmed by sequencing. Competent *E. coli* DH10 were then transformed with the pFASTBAC-hBA-STL plasmids, and cells were selected for kanamycin, gentamicin and tetracycline resistance. Bacmid DNA was isolated as described by Life Technologies. The presence of the hBR-STL sequence in the Bacmid DNA was confirmed by PCR using a PUC/M13 primer pair (forward primer, 5′-CCCAGTCACGACGTTGTA-AAACG-3′; reverse primer, 5′-AGCGGATAACAATTTCAC-ACAGG-3′). Sf9 insect cells were cultured and transfected with recombinant Bacmid DNA as described by Life Technologies. Recombinant baculovirus was harvested and used for the in-

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                                       TATAGAAGGTAGCCTGCAGGTACCGGTCCGGAATTCCCGGGTCGACCCAC   50
GCGTCCGGCGCGGGCGCGGGCGCGGGCGCGTGCGGGCTGCGAGCCGGGAGGCGGCGGCGGCGACGGCGACGGCGGCGGC  129

ATG GCG GAG AGC GAG GCC GAG ACC CCC AGC ACC CCG GGG GAG TTC GAG AGC AAG TAC TTC  189
Met Ala Glu Ser Glu Ala Glu Thr Pro Ser Thr Pro Gly Glu Phe Glu Ser Lys Tyr Phe   20
GAG TTC CAT GGC GTG CGG CTG CCG CCC TTC TGC CGC GGG AAG ATG GAG GAG ATC GCC AAC  249
Glu Phe His Gly Val Arg Leu Pro Pro Phe Cys Arg Gly Lys Met Glu Glu Ile Ala Asn   40
TTC CCG GTG CGG CCC AGC GAC GTG TGG ATC GTC ACC TAC CCC AAG TCC GGC ACC AGC TTG  309
Phe Pro Val Arg Pro Ser Asp Val Trp Ile Val Thr Tyr Pro Lys Ser Gly Thr Ser Leu   60
CTG CAG GAG GTG GTC TAC TTG GTG AGC CAG GGC GCT GAC CCC GAT GAG ATC GGC TTG ATG  369
Leu Gln Glu Val Val Tyr Leu Val Ser Gln Gly Ala Asp Pro Asp Glu Ile Gly Leu Met   80
AAC ATC GAC GAG CAG CTC CCG GTC CTG GAG TAC CCA CAG CCG GGC CTG GAC ATC ATC AAG  429
Asn Ile Asp Glu Gln Leu Pro Val Leu Glu Tyr Pro Gln Pro Gly Glu Leu Thr Ser Pro  100
GAA CTG ACC TCT CCC CGC CTC ATC AAG AGC CAC CTG CCC TAC CGC TTT CTG CCC TCT GAC  489
Arg Leu Ile Lys Ser His Leu Pro Tyr Arg Phe Leu Pro Ser Asp Leu Asp Ile Ile Lys  120
CTC CAC AAT GGA GAC TCC AAG GTC ATC TAT ATG GCT CGC AAC CCC AAG GAT CTG GTG GTG  549
Leu His Asn Gly Asp Ser Lys Val Ile Tyr Met Ala Arg Asn Pro Lys Asp Leu Val Val  140
TCT TAT TAT CAG TTC CAC CGC TCT CTG CGG ACC ATG AGC TAC CGA GGC ACC TTT CAA GAA  609
Ser Tyr Tyr Gln Phe His Arg Ser Leu Arg Thr Met Ser Tyr Arg Gly Thr Phe Gln Glu  160
TTC TGC CGG AGG TTT ATG AAT GAT AAG CTG GGC TAC GGC TCC TGG TTT GAG CAC GTG CAG  669
Phe Cys Arg Arg Phe Met Asn Asp Lys Leu Gly Tyr Gly Ser Trp Phe Glu His Val Gln  180
GAG TTC TGG GAG CAC CGC ATG GAC TCG AAC GTG CTT TTT CTC AAG TAT GAA GAC ATG CAT  729
Glu Phe Trp Glu His Arg Met Asp Ser Asn Val Leu Phe Leu Lys Tyr Glu Asp Met His  200
CGG GAC CTG GTG ACG ATG GTG GAG CAG CTG GCC AGA TTC CTG GGG GTG TCC TGT GAC AAG  789
Arg Asp Leu Val Thr Met Val Glu Gln Leu Ala Arg Phe Leu Gly Val Ser Cys Asp Lys  220
GCC CAG CTG GAA GCC CTG ACG GAG CAC TGC CAC CAG CTG GTG GAC CAG TGC TGC AAC GCT  849
Ala Gln Leu Glu Ala Leu Thr Glu His Cys His Gln Leu Val Asp Gln Cys Cys Asn Ala  240
GAG GCC CTG CCC GTG GGC CGG GGA AGA GTT GGG CTG TGG AAG GAC ATC TTC ACC GTC TCC  909
Glu Ala Leu Pro Val Gly Arg Gly Arg Val Gly Leu Trp Lys Asp Ile Phe Thr Val Ser  260
ATG AAT GAG AAG TTT GAC TTG GTG TAT AAA CAG AAG ATG GGA AAG TGT GAC CTC ACG TTT  969
Met Asn Glu Lys Phe Asp Leu Val Tyr Lys Gln Lys Met Gly Lys Cys Asp Leu Thr Phe  280
GAC TTT TAT TTA TAA                                                              984
Asp Phe Tyr Leu ***                                                              285

TAACAGAAACAACAACCTGCATGCTCACAATACCCAGACAGTCTACTAGCCAAAAGTCCTGTATGCATTCATTTATTCC 1063
TTGCTGGACAAACTCTGGAAGCAGCGTGTGAAACAGCGGGGAAGGGAAGAGCGGCGTGAGCGGAGGGAGTGTGATGAT 1142
TCCCAACCGAAAGCAGCTGTCTCGCCTTTAGAACGTGCAGCCTCTCCATGTCTGATTACAAACAGTCTCCACATTGCAG 1221
TTCCAATGGCCTGGACCGTAAGGATAAAGCCTGTAATATATGCAACTAGAATGTCTGCCTTTTCAACCCCGTATTATTT 1300
ATTGTATTTTATAGAGCTTTTCACTGGAAATCTACATAAATGTCAGTAAACCAAATAAAAGTTCATTTCCAAGGGGAAT 1379
CAGGAGCGAGCCACACCCGAATGGTAGAAAGATCTCAGGGTTAACTCTTTATTTTTGTAGTTTTATTATCTAAGGCACA 1458
GCCATTCTGTTCTCACTTGGTTCTGAGATAGTGGTGAGAACAGAGGATGAGTTGGGTCTGTTGGGGGAATCTGGACAC 1537
TTGTTTATTCTGACGGAGTTCACTTCTTCAGAACCTTCCTGAAATGAGCAGAAATTGTTCACTAGGTCTTCAGAATGGA 1616
CGTCCTTCTGCCAGAGACTTCCAGCGGGCGGCTCCAAAGGCCCAATGCAGAGGAGCCCGCGGAGCATGTGCTGAGGGAA 1695
GTCTGCCTGGTGAGGCTGGCAGGTGGGAGTCTAATGCAGTCAGGAGCATTTGCATGCAGTGGGTGGAGAGTCGGCCACC 1774
AAAGGACCGAGTTGCGCTCGGAATTTGAGCTGAATTCCACAGCCTTACTTTGTTTCCTGAAGTGATAGCCTACTAATGC 1853
TGGCAAGCAGATGCTTAATAGTAAATTTCTAAAATCCCCGGGTCTTTATCATTCAGTTTGTTCTGTGCACCTGAGGCGC 1932
TCAGCCGTGGGAGGACCATTTTGCGAGTGTAGCCCTGTTTCACTCGGATCAGGTTGGCACGGCCGCCTGCGTGTCTGTC 2011
CACCTCATCCCTCCGTGTATCTGAGGGAGTAAAGGTGAGGTCTTTATTGCTTCACTGCCTAATTTTCTCACCCACATTC 2090
GCTGAAGCGATGGAGAGTCGGGGGCCAGTAGCCAGCCAACCCCGTGGGGACCGGGGTTGTCTGTCATTTATGTGGCTGG 2169
AAAGCACCCAAAGTGGTGGTCAGGAGGGTCGCTGCTGTGGAAGGGGTCTCCGTTCTTGGTGCTGTATTTGAAACGGGTG 2248
TAGAGAGAAGCTTGTGTTTTTGTTTGTAATGGGGAGAAGCGTGGCCAGGCAGTGGCACGTGGCATCGCATGGTGGGCTC 2327
GGCAGCACCTTGCCTGTGTTTCTGTGAGGGAGGCTGCTTTCTGTGAAATTTCTTTATATTTTTCTATTTTTAGTACTGT 2406
ATGGATGTTACTGAGCACTACACATGATCCTTCTGTGCTTGCTTGCATCTTTAATAAAGACATGTTCCCGGC(63-A)  2541
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Figure 1 Nucleotide sequence and translation of hBR-STL-1

The stop codon is indicated by asterisks.

fection of fresh cultures of Sf9 cells. Cytosol was prepared from Sf9 cells expressing either hBR-STL or 6 × histidine-tagged (6 × His-tagged) hBR-STL. The 6 × His-tagged hBR-STL was purified further by Ni^{2+}-affinity chromatography [11]. The rBR-STL cDNA was expressed in Sf9 cells using the pFASTBAC-1 vector in a similar manner.

Northern-blot analysis

Total RNA was prepared from fresh or frozen rat tissues using STAT-60. RNA was prepared from whole frozen brains of male and female Sprague–Dawley rats, which were purchased from Harlan Bioproducts. Brains were obtained from rats at the ages of 1 day, 21 days, 8 weeks and 4 months. RNA was also isolated from adult Sprague–Dawley rat tissues at the time of killing. Northern-blot analysis using ^{32}P-labelled rBR-STL cDNA as a probe was performed as described previously [11]. Total RNA (20 μg) from the different tissues was resolved in a 1 % (v/v) formaldehyde/agarose gel and then transferred to a nylon membrane. Quantification of the RNA for the rat tissue Northern blots was performed by agarose-gel electrophoresis and densitometric scanning of the 28 S ribosomal RNA band. The levels of 28 S RNA in the samples were compared with known amounts of 28 S rRNA standard, and equivalent amounts of RNA were loaded. Transfer of the RNA was monitored by UV shadowing to ensure that the majority of the RNA was transferred to the nylon membrane. The rBR-STL cDNA was labelled with [^{32}P]dCTP as described previously. Hybridizations were performed for 1 h at 65 °C in Quickhyb (Stratagene) containing 1.25×10^6 c.p.m./ml [^{32}P]rBR-STL. The blots were washed twice for 15 min at room temperature in 2 × SSC containing 0.1 % (w/v) SDS, and then twice for 15 min in 0.1 × SSC containing

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hBR-STL    MAESEAETPSTPGEFESKYFEFHGVRLPPFCRGKMEEIA-NFPVRPSDVWI  50
hDHEAST    ..M.DDFL----------W..GIAFPTMG.RSETLRKVRDE.VI.DE..I.  39
hPPST      .ELIQDTS-------RPPLEYVK..P IKYFAEALGPLQ-S.QA..D.LL.  43
hMPST      .ELIQDTS-------RPPLEYVK..P IKYFAEALGPLQ-SSQA..D.LL.  43
hEST       .MN..LDY-------YE FE.V..ILMYKDFVKYWDNVE-A.QA..D.LV.  42
hST1B2     .LSPKDIL-------RKDLKLV..YPMTCAFASNW.K.E-Q.HS..D.IV.  43

hBR-STL    VTYPKSGTSLLQEVVYLVSQGADPDEIGLMNIDEQLPVLEYPQP-----GL  96
hDHEAST    L.......NW.A.ILC.MHSKG.AKW.QSVP.W.RS.WV.SEIG------Y  83
hPPST      S.......TWVSQILDMIY..G.LEKCHRAP.FMRV.F..FKAPGIPS- M  93
hMPST      N.......TWVSQILDMIY..G.LEKCNRAP.YVRV.F..VND GEPS- .  93
hEST       A.......TWVS I  MIYKEG.VEKCKEDV.FNRI.F..CRKENLMN- V  92
hST1B2     A.......TWVS IIDMILNDG.IEKCKRGF.TEKV.M..MTLPGLRTS I  94

hBR-STL    DIIKELTSPRLIKSHLPYRFLPSDLHNGDSKVIYMARNPKDLVVSYYQFHR 147
hDHEAST    TALS.SE....FSS...IQLF.KSFFSSKA....LM...R.VL..G.F.WK 134
hPPST      ETL.DTPA...L.T...LAL..QT LDQKV..V.V...A..VA....H.YH 144
hMPST      ETL.DTPP.........LAL..QT LDQKV..V.V......VA....H... 144
hEST       KQLD.MN...IV.T...PEL..ASFWEK.C.I..LC..A..VA..F.Y.FL 143
hST1B2     EQLEKNP...IV.T...TDL..KSFWENNC.M..L...A..VS....H.DL 145

hBR-STL    SLRTMSYRGTFQEFCRRFMNDKLGYGSWFEHVQEFWEHRMDSNVLFLKYED 198
hDHEAST    NMKFIKKPKSWE.YFEW.CQGTVL.....D.IHGWMPM.EEK.F.L.S..E 185
hPPST      MAKVHPEP..WDS.LEK..VGEVS....YQ....W..LSRTHP..Y F... 195
hMPST      MEKAHPEP..WDS.LEK..AGEVS....YQ....W..LSRTHP..Y F... 195
hEST       MVAGHPNP.SFP..VEK..QGQVP....YK..KSW..KGKSPR....F... 194
hST1B2     MNNLQPFP..WE.YLEK.LTGKVA.....T..KNW.KKKEEHPI...Y... 196

hBR-STL    MHRDLVTMVEQLARFLGVSCDKAQLEALTEHC-------------HQLVDQ 236
hDHEAST    LKQ TGRTI.KICQ...KTLEPEE NLILKNSSFQSMKENKMSNYSL.SVD 236
hPPST      .KENPKREIQKILE V.R.LPEETVDFMVQ.TSFKEMKKNPMTNYTTVPQE 246
hMPST      .KENPKREIQKILE.V.R.LPEETMDFMVQ.TSFKEMKKNPMTNYTTVPQE 246
hEST       LKE.IRKE.IK.IH..ERKPSEELVDRIIH.TSFQEMKNNPSTNYTT.P.E 245
hST1B2     .KENPKEEIKKII...EKNLNDEI DRIIH.TSFEVMKDNPLVNYTH.PTT 247

hBR-STL    CCNAEALPVGR-GRVGLWKDIFTVSMNEKFDLVYKQKMGKCDLTFDFYL*  284
hDHEAST    YVVDK-AQLL.K.VS.D..NH...AQA.D..KLFQE..ADLPRELFPWE*  285
hPPST      FMDHSIS.FM.K.MA.D..TT...AQ..R..AD.AE..AG.S.S.RSE *  295
hMPST      LMDHSIS.FM.K.MA.D..TT...AQ..R..AD.AE..AG.S.S.RSE *  295
hEST       IM QKLS.FM.K.IT.D..NH...AL.....KH.E.Q.KEST.K.RTEI*  294
hST1B2     VMDHSKS.FM.K.TA.D..NY...AQ.....AI.ETE.S.TA.Q.RTEI*  296
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Figure 2 Alignment of the amino acid sequence of hBR-STL-1 with those of five other human cytosolic SULTs

The sequences used in the comparison were hST1B2 [10], hP-PST-1 [25], hMPST [26], hDHEA-ST [21] and hEST [16]. The amino acid sequences of the SULTs were aligned using the MacVector Sequence analysis software. The initial methionine residue of the hST1B2 sequence is denoted as the first amino acid. Short gaps, represented by dashes, were inserted into the sequence comparisons to optimize the alignments. Dots represent amino acids identical with those of hBR-STL. Asterisks indicate the stop codons.

0.1 % SDS at 60 °C. Autoradiography was performed at −70 °C with an intensifying screen.

Immunoblot analysis

Several attempts to raise polyclonal antibodies against purified His-tagged hBR-STL in rabbits were unsuccessful. Therefore polyclonal antibodies were raised against pure His-tagged hBR-STL in chickens, and polyclonal IgY antibodies were purified by Ni^{2+}-affinity chromatography (Lampire Biologicals, Pipersville, PA, U.S.A.). To raise an anti-(BR-STL) antibody, His-tagged hBR-STL protein obtained following the Ni^{2+}-affinity procedure was purified further by SDS/PAGE and electroelution of the His-tagged hBR-STL protein from gel slices using a Protean II electroelution system, as described by Wang et al. [11]. For immunoblot analysis, adult rat brain cytosolic proteins were resolved by SDS/PAGE in a 10 % polyacrylamide gel and electrotransferred on to nitrocellulose paper. The chicken anti-(hBR-STL) sera was diluted 1:2500 and incubated with the nitrocellulose filter for 1 h. Rabbit anti-chicken IgY peroxidase conjugate (Promega) was used as a secondary antibody, and immunoconjugates were revealed by chemiluminescence using a Luminol chemiluminescence substrate kit.

RESULTS

Isolation and characterization of hBR-STL cDNA

The original hBR-STL cDNA sequences were isolated from human pancreatic islet RNA during differential display to identify transcripts enriched in human islet cells relative to pancreatic exocrine tissue [7]. Alignment of these partial sequences to known human SULT sequences suggested that these were STL proteins. Northern-blot analysis of RNA isolated from various human tissues indicated that the message for this STL was highly expressed in brain tissue, although no message was detectable in pancreatic tissue (results not shown). These BR-STL partial cDNAs isolated from the pancreatic library were ultimately shown to possess unspliced intronic sequence. The pancreatic STL cDNA was used to screen a human brain SuperScript pCMV-Sport plasmid cDNA library under high-stringency conditions. Three positive clones were isolated following the screening of approx. 100000 separate bacterial colonies. Figure 1 shows the nucleotide sequence and translation of hBR-STL-1, the longest of the three cDNAs. The hBR-STL-1 cDNA contains an 852-nt open reading frame encoding a 284-amino-acid protein with a calculated molecular mass of 33083 Da. The other two cDNAs were truncated forms of hBR-STL-1, which possessed

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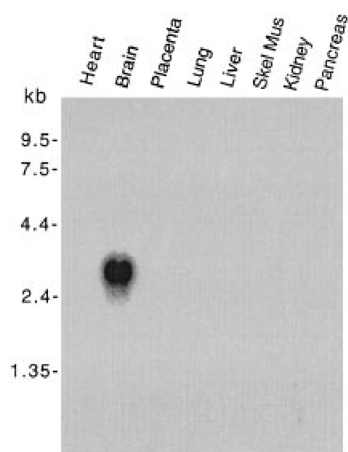

Figure 3 Northern blot analysis of RNA isolated from different human tissues using the hBR-STL cDNA as a probe

A commercial multiple tissue RNA blot (Clontech) containing RNA isolated from eight different human tissues was probed with ^{32}P-labelled hST1B2 cDNA. Each lane of the blots contained approx. 2 μg of poly(A)$^{+}$ RNA. Skel Mus, skeletal muscle.

nucleotide sequences identical with hBR-STL-1. Compared with the nucleotide sequence, these cDNAs encoded bases 594–1006 and 214–993.

Figure 2 shows the alignment of the amino acid sequence of hBR-STL-1 with the sequences of five other human cytosolic SULTs. hBR-STL-1 is 49 %, 54 %, 51 % and 53 % similar to, and 30 %, 34 %, 34 %, 33 % and 36 % identical with, the sequences of human DHEA-ST, EST, P-PST-1, ST1B2 and M-PST respectively. The translated product of the hBR-STL-1 transcript also contains several sequences that are conserved in many SULTs [12]. These include the 5′ TYPKSGT sequence (residues 52–58) involved with 3′-phosphoadenosine 5′-phosphosulphate (PAPS) binding, the putative active-site histidine residue (residue 111), the internal 'YGSWXXH' motif (residues 172–178; 'X' denotes 'any other residue') and 3′ GXXWKXXFTV, reported to also be involved with PAPS binding [13,14].

Northern-blot analysis of RNA from human tissues

Figure 3 shows the Northern-blot analysis of the expression of hBR-STL message in several human tissues. The original cloning of an hBR-STL cDNA was carried out from human pancreatic RNA; however, no message was detectable in poly(A)$^{+}$ RNA

```
Human liver                          GGCTCGCAACCCCAAGGATCTGGTGGTGT   29
hBR-STL      AGACTCCAAGGTCATCTATATGGCTCGCAACCCCAAGGATCTGGTGGTGT  550

Human liver  CTTATTATCAGTTCCACCGCTCTCTGCGGACCATGAGCTACCGAGGCACC   79
hBR-STL      CTTATTATCAGTTCCACCGCTCTCTGCGGACCATGAGCTACCGAGGCACC  600

Human liver  TTTCAAGAATTCTGCCGGAGGTTTATGAATGATAAGCTGGGCTACGGCTC  129
hBR-STL      TTTCAAGAATTCTGCCGGAGGTTTATGAATGATAAGCTGGGCTACGGCTC  650

Human liver  CTGGTTTGAGCACGTGCAGGAGTTCTGGGAGCACCGCATGGACTCGAACG  179
hBR-STL      CTGGTTTGAGCACGTGCAGGAGTTCTGGGAGCACCGCATGGACTCGAACG  700

Human liver  TGCTTTTTCTCAAGTATGAAGACATGCATCGGGACCTGGTGACGATGGTG  229
hBR-STL      TGCTTTTTCTCAAGTATGAAGACATGCATCGGGACCTGGTGACGATGGTG  750

Human liver  GAGCAGCTGGCCAGATTCCTGGGGGTGTCCTGTGACAAGGCCCAGCTGGA  279
hBR-STL      GAGCAGCTGGCCAGATTCCTGGGGGTGTCCTGTGACAAGGCCCAGCTGGA  800

Human liver  AGCCCTGACGGAGCACTGCCACCAGCTGGTGGACCAGTGCTGCAACGCTG  329
hBR-STL      AGCCCTGACGGAGCACTGCCACCAGCTGGTGGACCAGTGCTGCAACGCTG  850

Human liver  AGGCCCTGCCCGTGGGCCGGGCACATTGCGTCTTTGCTCGGAAGATCTTC  379
hBR-STL      AGGCCCTGCCCGTGGGCCGG------------------------------  870

Human liver  TTGAGTTGGTGAGAATGCAGGGCCGAGTAGCCAGCTGCATTGCTTAGATT  429
hBR-STL      --------------------------------------------------  870

Human liver  TGATTCATGTCACAGCATAAATTGTCTTGATTCCAAATCCTAAACCAGGA  479
hBR-STL      -----------------------------------------------GGA  873

Human liver  AGAGTTGGGCTGTGGAAGGACATCTTCACCGTCTCCATGAATGAGAAGTT  529
hBR-STL      AGAGTTGGGCTGTGGAAGGACATCTTCACCGTCTCCATGAATGAGAAGTT  923

Human liver  TGACTTGGTGTATAAACAGAAGATGGGAAAGTGTGACCTCACG         572
hBR-STL      TGACTTGGTGTATAAACAGAAGATGGGAAAGTGTGACCTCACGTTTGACT  973
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Figure 4 Comparison of the RT-PCR product generated from human liver RNA using hBR-STL-1-specific primers

A primer pair specific for the amplification of hBR-STL was used to generate the hBR-STL product using human liver RNA as a template. The RNA was prepared from the liver of a 14-year-old white female using STAT-60. The only RT-PCR product generated from the liver RNA was isolated, subcloned into pCR2.1, and sequenced. The amino acid sequences of the SULTs were aligned using the MacVector Sequence analysis software. A gap, represented by dashes, was inserted into the sequence comparison to optimize the alignment. The sequences of the PCR primers are shown underlined.

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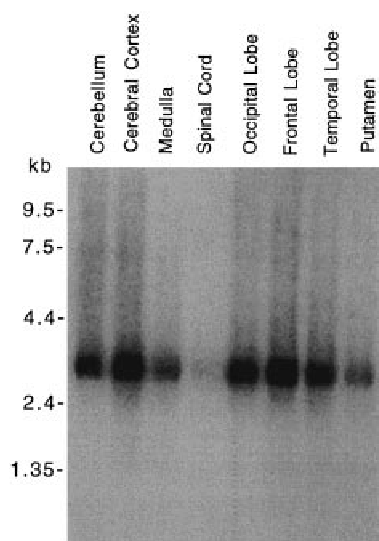

Figure 5 Localization of hBR-STL expression in human brain was investigated by Northern-blot analysis of poly(A)$^+$ RNA isolated from eight different brain regions

A commercial multiple tissue RNA blot (Clontech) containing RNA isolated from eight different human brain regions was probed with ^{32}P-labelled hBR-STL cDNA. Each lane of the blots contained approx. 2 μg of poly A$^+$ RNA.

isolated from that tissue. hBR-STL message was readily detectable in poly(A)$^+$ RNA isolated from human brain, but not from heart, placenta, lung, liver, skeletal muscle, kidney or pancreas. Although hBR-STL message could not be detected in Northern-blot analyses of pancreas or liver poly(A)$^+$ RNA, reverse transcriptase (RT)-PCR of liver RNA with hBR-STL-specific primers generated only one product that was 126 bp longer than the PCR product obtained with the hBR-STL cDNA as a template. Figure 4 shows that the RT-PCR product generated from human liver RNA was identical with the sequence of the brain cDNA, except that it contains a 126-bp insert located at the same position as intron 7 of several human SULT genes [12]. Several in-frame termination codons are present in the putative intronic sequence. Subsequent analysis of several human pancreatic cell lines (CFPAC-1, PANC-1 and CAPAN-1) by RT-PCR also detected only amplification products with the additional 126-bp sequence located at the site of intron 7 (results not shown). These results suggest that BR-STL message may be expressed in several tissues; however, in some tissues the mRNA might not be properly processed to generate the appropriate message that can be transcribed. Furthermore, it is possible that BR-STL message is selectively expressed in islet cells, which contribute only a small fraction of the poly(A)$^+$ RNA isolated from pancreas.

hBR-STL expression in human brain was investigated by Northern-blot analysis of poly(A)$^+$ RNA isolated from eight different brain regions (Figure 5). The expression of hBR-STL message was highest in the cerebral cortex and frontal lobe, and slightly less in the cerebellum, occipital lobe and temporal lobe. Levels of hBR-STL message were relatively low in the medulla and putamen, and lowest in the spinal cord.

Isolation of the rat BR-STL cDNA

The rBR-STL cDNA was isolated from a rat brain λZAP cDNA library using the coding region of hBR-STL as a probe. Seven

```
hBR-STL         MAESEAETPSTPGEFESKYFEFHGVRLPPFCRGKMEEIANFPVRPSDVWIV  50
rBR-STL                  G                          D  D                50

hBR-STL         TYPKSGTSLLQEVVYLVSQGADPDEIGLMNIDEQLPVLEYPQPGLDIIKEL  96
rBR-STL                                                             96

hBR-STL         TSPRLIKSHLPYRFLPSDLHNGDSKVIYMARNPKDLVVSYYQFHRSLRTMS 147
rBR-STL                                                            147

hBR-STL         YRGTFQEFCRRFMNDKLGYGSWFEHVQEFWEHRMDSNVLFLKYEDMHRDLV 198
rBR-STL                                            A               198

hBR-STL         TMVEQLARFLGVSCDKAQLEALTEHCHQLVDQCCNAEALPVGRGRVGLWKD 236
rBR-STL                             S I                            236

hBR-STL         IFTVSMNEKFDLVYKQKMGKCDLTFDFYL*    284
rBR-STL                                           284
```

Figure 6 Comparison of the translation products of hBR-STL-1 and rBR-STL-1

The amino acid sequences of the SULTs were aligned using the MacVector Sequence analysis software. The initial methionine residue of both sequences is denoted as the first amino acid. Asterisks indicate the stop codons.

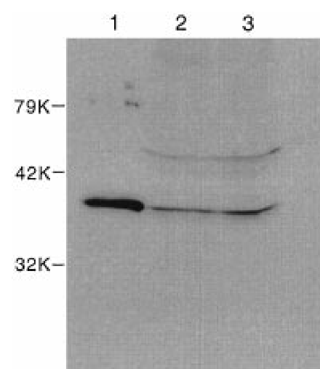

Figure 7 Immunoblot analysis of male and female rat brain cytosol with chicken anti-(rBR-STL) IgY antibodies

Cytosol was prepared from the whole brains of adult male and female Sprague–Dawley rats (Harlan). Cytosolic protein was resolved in an SDS/10% polyacrylamide gel and electrotransferred on to a nitrocellulose membrane. The primary antibody used was chicken anti-(hBR-STL) IgY (1:2500 dilution), and the secondary antibody was a rabbit anti-chicken IgY peroxidase conjugate. Lane 1 contained cytosol (2 μg) from Sf9 insect cells expressing rBR-STL; lanes 2 and 3 contain cytosol (200 μg) from male and female rat brains respectively. Molecular mass markers for reference are shown on the left (K = kDa).

cDNAs were ultimately obtained from screening of approx. 200000 phage in the rat brain library. The longest cDNA, rBR-STL-1, was 2231 bp in length, possessed a 32-bp 5′-non-translated region, an 852-bp open reading frame encoding a 284-amino-acid protein, and a 1326-bp 3′-non-translated region followed by a poly(A)$^+$ tail. The other rBR-STL cDNAs ranged from 2183 to 273 bp in length. The 2183-bp cDNA was identical in nucleotide sequence with hBR-STL-1, except that it was 49 nucleotides shorter at the 5′-end. Figure 6 shows the comparison of the translation products of hBR-STL-1 and rBR-STL-1. The open reading frames of both hBR-STL and rBR-STL cDNAs encode 284-amino-acid proteins with sequences that are 98% identical and 99% similar. The nucleotide sequences are $>91\%$ identical in the coding regions of the human and rat BR-STL cDNAs.

Immunoblot analysis of rBR-STL in rat brain

hBR-STL was expressed in Sf9 insect cells using the FastBacHTB vector, which incorporates six histidine residues at the N-terminal end of the protein. His-tagged hBR-STL protein was purified by

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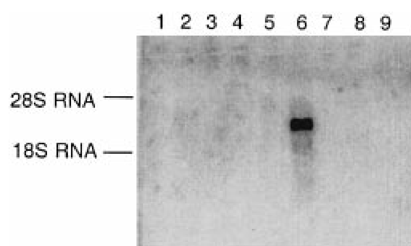

Figure 8 Northern-blot analysis of RNA isolated from different rat tissues using the rBR-STL cDNA as a probe

Total RNA was isolated from different tissues of an adult male Sprague–Dawley rat using STAT-60. Total RNA (20 μg) from the different tissues was resolved in a 1% (v/v) formaldehyde/agarose gel and transferred to a nylon membrane. The membrane was probed with ^{32}P-labelled rBR-STL cDNA. The lanes contain RNA as follows: lane 1, liver; lane 2, kidney; lane 3, spleen; lane 4, heart; lane 5, lung; lane 6, brain; lane 7, small intestine; lane 8, testis. Lane 9 contained 28 S RNA standard.

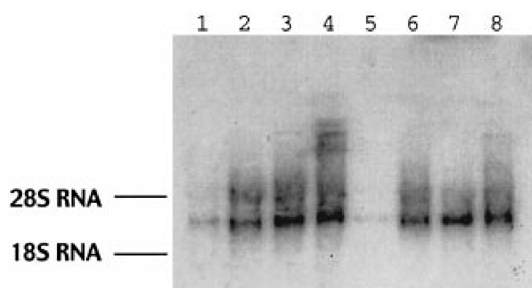

Figure 9 Northern-blot analysis of the expression of rBR-STL cDNA in rat brains from animals of different age and sex

Total RNA (20 μg) from the different brain samples was resolved in a 1% (v/v) formaldehyde/agarose gel and transferred to a nylon membrane. The membrane was probed with ^{32}P-labelled rBR-STL cDNA. The lanes contain rat brain RNA as follows: lane 1, 1-day-old male; lane 2, 21-day-old male; lane 3, 8-week-old male; lane 4, 4-month-old male; lane 5, 1-day-old female; lane 6, 21-day-old female; lane 7, 8-week-old female; lane 8, 4-month-old female.

Ni^{2+}-affinity chromatography and electroelution for use as an antigen, and as a standard in the immunoblotting procedures. Initial attempts to raise polyclonal antibodies in rabbits using procedures that were effective for other cytosolic SULTs [11,15–17] were unsuccessful. Also attempts to raise rabbit antibodies to hBR-STL by commercial laboratories were unsuccessful. Therefore polyclonal IgY antibodies were raised against pure His-tagged hBR-STL in chickens. Figure 7 shows that the chicken anti-(hBR-STL) IgY antibodies react with a protein in whole-brain cytosol prepared from adult male and female rats, which co-migrates with expressed rBR-STL. The chicken anti-(hBR-STL) IgY antibodies do not react with bacterially expressed P-PST1, M-PST, EST, ST1B2 or DHEA-ST (results not shown). This result demonstrates that the rBR-STL protein is expressed in rat brain.

Northern-blot analysis of the expression of rBR-STL

For comparison with the tissue expression of the hBR-STL message, Northern-blot analysis of total RNA (5 μg) from eight female rat tissues was performed using the rBR-STL cDNA as a probe. rBR-STL expression was readily detected in RNA from whole rat brain, but not in RNA isolated from liver, kidney, spleen, heart, lung, small intestine or testis (Figure 8).

The developmental expression of rBR-STL message in rats was investigated to determine whether levels of rBR-STL message in male and female Sprague–Dawley rats varied with age. Figure 9 shows that relatively low levels of rBR-STL message were detectable in total RNA from 1-day-old male and female rat brains. Message levels in whole rat brain increased significantly in both sexes by 21 days of age, and remained high in 2- and 4-month-old rats. rBR-STL message levels increase significantly shortly after birth in both male and female rats. No obvious differences were observed in message levels between the sexes.

DISCUSSION

This study describes the isolation, expression and characterization of the cDNAs for a novel form of cytosolic protein in human and rat brain, which is structurally related to the cytosolic SULTs. These proteins have tentatively been termed hBR-STL and rBR-STL, because of their similarity to the SULT gene family. This identification has not been confirmed by the demonstration of sulphation activity with the expressed BR-STL proteins. The BR-STL cDNAs isolated from both rat and human brain cDNA libraries have been expressed in both *E. coli* and Sf9 insect cells, which in our laboratory have been used previously to generate enzymically active SULTs [11,16,17]. Approximately 20 prototypical SULT substrates have been tested as substrates with the expressed BR-STLs; however, sulphation activity has not been detected with any of these substrates. The possibility exists that the BR-STL enzymes may have a very selective substrate reactivity, or that the functional enzymes may be active as part of a multi-enzyme complex.

The lack of detectable sulphation activity by either of the expressed BR-STLs has limited the characterization of these proteins. Identification of the BR-STLs as members of the SULT family is on the basis of sequence similarities. Both the rBR-STL and hBR-STL amino acid sequences possess the conserved P-loop structural motif (5′-TYPKSGT), which is proposed to interact with the 5′-phosphate of PAPS in the active site of the SULTs [14]. Both BR-STLs also contain the histidine residue hypothesized to act as a base in the active site of the SULT gene family [14]. An important difference in the BR-STLs from the mammalian SULTs is in a conserved site at the 3′-end of the sequence (246–254 of BR-STL), proposed to be involved in interaction with the 3′-phosphate group of PAPS [13,14]. The BR-STLs have an arginine, rather than a lysine, residue immediately preceding the GXXWKXXFTV motif, which is conserved among the other human SULTs [18]. The BR-STLs also have a 13-amino-acid gap in their alignment with the other human SULT primary protein structures, which occurs several amino acids before this conserved motif (Figure 2). Although the BR-STLs are apparently members of the SULT gene family, differences in the C-terminal ends of the BR-STL proteins as compared with other human cytosolic SULTs might have important effects on PAPS binding and substrate specificity.

An interesting feature of the BR-STLs is the high degree of sequence homology shared between the rat and human proteins. Six amino-acid differences were observed between these sequences, and three of these were conserved substitutions. This is the highest sequence similarity observed between two SULTs, or, in this case, SULT-like proteins, from different species. The inability to raise an antibody in rabbits with the pure expressed hBR-STL protein also suggests a high degree of similarity of the rabbit protein to the rat and human proteins. The SULT gene family in general shows approx. 70–75% sequence similarity

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among orthologous enzymes in different species. The high degree of sequence similarity suggests a conserved function among species.

Tissue-specific expression of members of the SULT gene family is important in understanding their biological and physiological properties [1]. Several SULTs have been identified in human brain [3,5,6]; however, these SULTs are also found at high levels in other tissues. The phenol SULTs, P-PST-1 (ST1A1) and M-PST (ST1A3), which have been localized in brain [4,6], are also expressed in the liver and/or intestine at high levels. Hydroxysteroid SULTs identified in rat brain [19,20] are also expressed in liver [21,22]. The functions of the SULTs in brain are poorly understood, although sulphation and inactivation of monoamine neurotransmitters and enkephalins have been proposed as potential roles for the phenol SULTs. The hydroxysteroid SULTs might be involved in regulating the activity of neurosteroids in both the central and peripheral nervous systems [23,24]. The selective expression of high levels of the BR-STLs in brain tissue, and the high degree of sequence conservation between humans and rats, suggests an important role for these proteins in the central nervous system.

The BR-STLs represent a group of proteins that are related to the SULT family, the genes of which exhibit a high level of expression in the cortical regions of the brain. The rat and human orthologues possess a much greater sequence homology than that observed with other SULTs. The physiological role of this family of SULTs is not known; however, these SULTs are unlikely to be involved primarily in drug or xenobiotic sulphation, but more likely will be shown to have a physiological function in the brain. The physiological functions of the cytosolic SULTs are not as extensively studied as their roles in bioactivation or xenobiotic conjugation. Therefore the selective localization of BR-STL gene expression, and the high level of sequence conservation among species, suggests an important conserved function.

This research was supported in part by PHS grant GM38953 (to C.N.F.).

Received 1 October 1999/25 November 1999; accepted 12 January 2000

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