Expression and Localization of Cytosolic Sulfotransferase (SULT) 1A1 and SULT1A3 in Normal Human Brain

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ABSTRACT:

Cytosolic sulfotransferases (SULTs) are a family of Phase II drug-metabolizing enzymes that catalyze the transfer of a sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate to endogenous and xenobiotic compounds. Several SULT isoform messages have been detected in the human brain; however, protein expression patterns have not been characterized. Immunoblot analysis of the SULT1A1 and 1A3 isoforms was carried out with cytosolic fractions isolated from superior temporal gyrus, hippocampus, cerebellum, occipital pole, frontal pole, and temporal pole regions of normal adult human brains. SULT1A1 expression was highest in cytosolic fractions isolated from cerebellum, occipital, and frontal lobes, whereas, SULT1A3 expression was highest in cytosol from superior temporal gyrus, hippocampus, and temporal lobe. SULT1A1 and SULT1A3 immunoreactivities were found in both neurons and glial cells by immunohistochemical analysis in all brain regions studied. SULT1A1 is known to catalyze the metabolism of small phenols, whereas SULT1A3 sulfates catecholamine neurotransmitters. Because SULT1A1 and 1A3 have distinct substrate specificities, the differences in expression pattern and cellular localization of the SULT1A isoforms are probably associated with the distribution and function of their selective substrates in the different brain regions.

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ABBREVIATIONS: SULT, cytosolic sulfotransferase; MNTs, monoamine neurotransmitters; ASD, autism spectrum disorders; PBE, phosphate-buffered saline with EDTA; TBST, Tris-buffered saline in Tween 20.
therapeutic studies, different SULT1A allozymes have been shown to exhibit substantial differences in catalysis of xenobiotic conjugation (Rafatogianis, 1997; Bardakci et al., 2008). In particular, these polymorphisms have been implicated in altered metabolism of 4-hydroxytamoxifen (Gjerde et al., 2008) and changes in bioactivation of pathological electrophiles (Falany, 2004). Differences in tamoxifen metabolism may have considerable clinical consequences because they are associated with therapeutic outcome and should be considered when choosing a treatment regimen (Nowell and Falany, 2006).

Alterations in SULT1A expression may also affect normal brain physiology. More recently, genetic linkage of the SULT1A locus and a predisposition to autism spectrum disorders (ASD) has been reported (Dooley et al., 1993; Kumar et al., 2008; Weiss et al., 2008). Furthermore, individuals with ASD have decreased expression of SULT1A isoforms in platelets and in the gastrointestinal tract (Waring et al., 1996; Waring and Klovzra, 2000; Whiteley and Shattuck; Kern et al., 2004). These decreases in SULT1A catalytic activity are also associated with increases in circulating levels of putative substrates such as neurotransmitters and catechols (Whiteley and Shattuck, 2002). Currently, the levels of SULT1A isoforms in the brains of individuals with ASD is unknown. Identifying the normal expression profiles for the SULT1A isoforms in human brain will assist in clarifying the functions of the SULTs in normal human brain as well as lead to progress in understanding the pathological outcomes when these isoforms are aberrantly expressed.

Materials and Methods

Immunohistochemistry. Paraffin blocks of human brain regions were obtained from the Tissue Procurement Service of the Comprehensive Cancer Center of the University of Alabama at Birmingham (UAB) (Birmingham, Alabama). The blocks were cut into 5-μm serial slices, mounted to microscope slides coated with poly-L-lysine, and oven-dried by the UAB Comparative Histology Laboratory. Slices were deparaffinized in xylene and rehydrated in alcohol baths (absolute, 95%, 70%) then rinsed in dH2O and Tris wash buffer (0.5 M Tris, 0.15 M NaCl, 0.01% Triton X-100, pH 7.6). Optimal staining was achieved without the use of antigen retrieval techniques. Endogenous peroxidase activity was quenched with 3% H2O2 for 10 min then slides were blocked in 1% goat serum for 1 h in humidity chambers. Primary antibody, either rabbit anti-human SULT1A1 IgG or a polyclonal rabbit anti-human SULT1A3 peptide, was diluted in PBE (phosphate-buffered saline with 500 mM EDTA, 1% bovine serum albumin, pH 7.6) at a ratio of 1:100 and incubated on slides for 1 h in humidity chambers. The rabbit anti-SULT1A1 antibody was raised against pure SULT1A1 and due to the high sequence identity between the SULT1A isoforms reacts with both isoforms (Falany, 2004). A selective rabbit polyclonal anti-human SULT1A3 antibody was generated using a peptide with the sequence EVNDPGPESGLTLL (83–97) as the immunogen (Open Bio-systems, Huntsville, AL). Monoclonal antibodies against neuronal nuclei and glial fibrillary acid protein were purchased from Millipore Bioscience Research Reagents (Temecula, CA) and diluted in PBE according to the manufacturer’s specifications. Negative control slides were incubated in preimmune or 1% goat serum instead of primary antibody. Slides were washed two times in Tris wash buffer for 5 min before incubation for 10 min with anti-rabbit biotin conjugate (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted 1:500 in PBE. Slides were washed twice in Tris wash buffer for 5 min then incubated for 5 min with streptavidin-labeled conjugate (Jackson ImmunoResearch Laboratories Inc.), diluted 1:500 in PBE. Slides were washed twice in Tris wash buffer for 5 min, and the liquid 3,3’-diaminobenzidine (DAB kit; Biogenex, San Ramon, CA) was used to develop the slides according to the manufacturer’s instructions. Slides were rinsed in dH2O then counterstained in hematoxylin for 45 s. After rinsing in H2O for 4 min, slides were dehydrated in graded alcohols and xylene and then permanently coverslipped. Stained slides were imaged using a Zeiss Axiosstar microscope with camera (Carl Zeiss Microimaging, Inc., Thornwood, NJ) and corresponding AxioVision software (Carl Zeiss Microimaging, Inc.). Tissues stained for immunohistochemical analysis using the SULT1A1 Ig antibody were compared with serial sections of the same tissue stained with the SULT1A3 peptide antibody to determine which isoforms were expressed in distinct cell types.

Immunoblot Analysis. Frozen sections of different regions of normal adult human brains were obtained from the UAB Alzheimer’s Disease Research Center. Tissue from normal superior temporal gyrus, hippocampus, cerebellum, occipital pole, frontal pole, and temporal pole was used to prepare cytosolic fractions. Approximately 200 mg of tissue was homogenized in 1 ml of ice-cold phosphate buffer (10 mM KH2PO4, 1 M dithiothreitol, 10% glycerol, pH 7.4) then centrifuged at 100,000g for 1 h. Supernatant fractions were aliquoted and stored at −80°C. Total cytosolic protein was determined by the Bradford assay (Bio-Rad, Hercules, CA) using gamma globulin as a standard. For immunoblots, 100 to 200 μg of cytosolic protein was heated to 95°C for 5 min in sample loading buffer containing SDS and β-mercaptoethanol. Bacterially expressed purified human SULTs were used as positive controls, to generate standard curves for isoform quantitation and to standardize immunoreactivity (data not shown). Samples were loaded onto a 10% SDS/polyacrylamide gel and electrophoresed at 200 V for 45 min. Proteins resolved in polyacrylamide gels were transferred to nitrocellulose membranes via semi-dry transfer (Bio-Rad) at 12 V for 20 min in semidy transfer buffer (48 mM Tris, 39 mM glycine, 20% methanol, pH 9.2). Membranes were blocked from 4 h to overnight at 4°C in absolute SEA Block (Thermo Fisher Scientific, Waltham, MA). After blocking, membranes were washed three times in Tris-buffered saline in Tween 20 (TBST) before overnight incubation at 4°C with the primary antibody, diluted 1:1000 in 0.1% milk in TBST. Membranes were washed twice for 5 min in TBST at room temperature then incubated in goat anti-rabbit horseradish peroxidase diluted 1:50,000 in 0.1% milk in TBST for 1 h at room temperature. Immunoblots were developed in West Pico or Femto SuperSignal (Thermo Fisher Scientific) and exposed to autoradiograph film. Relative quantitation of bands was performed using Un-Scan-IT (version 6.1) software (Silk Scientific Inc., Orem, UT) for densitometry and normalizing data to the ratio of SULT1A1 to SULT1A3 immunoreactivity. The ratios were corrected to represent actual protein expression of the isoforms because SULT1A1 is more immunoreactive than SULT1A3 for the same amount of protein using the SULT1A1 IgG antibody.

Results and Discussion

Specific Immunoreactivity. SULT1A1 and SULT1A3 protein expression was detected predominantly with rabbit anti-human SULT1A1 IgG (Fig. 1). The rabbit anti-SULT1A3 peptide antibody showed only slight reactivity to SULT1A1-expressed human protein (Fig. 1). Only negligible cross-reactivity to other SULT isoforms was shown with either antibody, except for SULT1C2 (Fig. 1). Although SULT1C2 did show cross-reactivity for the SULT1A3 peptide antibody, the isoforms are distinguished easily by their electrophoretic migration patterns. Despite the cross-reactivity, SULT1C2 was not
detected in the brain sections and the SULT1A3 antibody was not used in the brain immunoblot experiments. The anti-SULT1A1 IgG detects both SULT1A1 and SULT1A3 proteins with similar affinity due to the high degree of sequence identity (93%) (Falany, 2004) between these isoforms. Because the anti-SULT1A1 antibody detects both SULT1A proteins and shows no significant cross-reactivity, it was used to quantify the relative amount of the proteins expressed in cytosol isolated from normal human brain sections.

**Expression Patterns of SULTs 1A1 and 1A3.** Immunoreactive SULT1A1 and SULT1A3 were detected in all regions of the normal human brains analyzed (Fig. 2). However, the expression of these two isoforms varies between brain sections and among brains. Figure 2 depicts an inverse expression pattern between SULT1A1 and SULT1A3 in select brain regions. In general, in sections where SULT1A1 showed higher protein expression levels, SULT1A3 showed lower protein expression, and where SULT1A3 expression was higher, SULT1A1 expression was lower. This was apparent in all regions except the frontal lobe, which showed similar immunoreactivity for SULT1A1 and SULT1A3. In general, SULT1A1 was most highly expressed in the cerebellum and occipital lobe, whereas SULT1A3 demonstrated highest expression in the superior temporal gyrus, hippocampus, and temporal lobe. In the frontal lobe, both isoforms were highly expressed.

**Cellular Localization of SULTs 1A1 and 1A3.** SULT1A1 and SULT1A3 are expressed in multiple cell types in the human brain (Fig. 3). Immunohistochemical analysis of normal frontal and temporal lobes shows the expression of these isoforms in both neurons and in glial cells. The antibodies to neuronal nuclei and glial fibrillary acid protein were used as markers for these cell types, respectively. However, the SULT1A isoforms were not expressed uniformly in all neurons and glial cells. As shown in Fig. 3, only select neurons are immunoreactive for SULT1A1 in frontal and temporal lobes. Figure 3 also shows that SULT1A staining is observed in microglial and oligodendrocyte cells. Immunoreactivity for the SULT1A isoforms is localized to the cytosol in the subcellular compartments of these cell

dtypes. This is relevant because some of the human cytosolic SULT isoforms (SULT2B1b) have been reported to have inducible nuclear localization in non-neural tissues (Falany et al., 2006). Neuronal localization of the phenol SULT isoforms was first reported by Zou et al. (1990) using a nonspecific anti-SULT1A antibody. These investigators did not report the differential expression of the two isoforms. Whereas the SULT1A isoforms are expressed in neurons, this report demonstrates that SULT1A expression is not limited to neurons, and expression can be detected in multiple regions of the human brain. In the temporal lobe, SULT1A3 is expressed predominantly in microglia and oligodendrocytes, and this may account for the increased immunoreactivity of SULT1A3 in this brain region. Differential immunoreactivity may be associated with epitope availability, substrate distribution, neuronal subtype, and synaptic status. Western blot analysis of cultured human SH-SY5Y neurons and CCF-STTG1 astrocytes was performed to further examine cell type-specific expression of the SULT1A isoforms. SULT1A1 and SULT1A3 protein expression was not detected in these cultured cells (data not shown), implying that the SULT1A isoforms are most likely expressed in specific neural subtypes rather than constitutively in all cells of the human brain.

This report demonstrates that SULT1A1 and SULT1A3 are differentially expressed in multiple regions of normal adult human brain.
Furthermore, it has been shown that the SULT1A isoforms are located in the cytosol of multiple cell types in regions of normal human brain. A varying pattern of expression between brain regions is probably due to the different physiological functions of the isoforms. SULT1A1 is a xenobiotic-conjugating enzyme with a broad substrate range, whereas SULT1A3 is responsible for the regulation of the rapidly fluctuating levels of neurotransmitters. A better understanding of the specific cell types that express the SULT1A isoforms needs to be elucidated. By exploring the role of the SULT1A isoforms in cell culture models, multiple regions of normal fetal brain, as well as comparison of expression patterns of the SULT isoforms in regions of non-normal brains, e.g., Alzheimer’s disease and schizophrenia.

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References


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