Identification and Localization of Two Hydroxysteroid Sulfotransferases in the Human Brain

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Hydroxysteroid sulfotransferase (SULT2) is thought to be a key enzyme in the synthesis of neurosteroid sulfates, which are known to act as potent regulators of neuronal activity within the brain. We detected two SULT2 mRNAs (SULT2A1 and SULT2B1b) in the human brain. We isolated their cDNAs, and determined the location of their expression in the brain by Northern blot hybridization. Exclusive SULT2A1 expression was detected in the thalamus and hypothalamus, whereas SULT2B1b expression was detected throughout the brain in varying amounts, reaching levels two to three times greater in some regions than in others. These findings further our understanding of the physiological role of SULT2 enzymes within the human brain, particularly how they affect neurosteroid metabolism, and thus, modulate neuronal activity within the brain.

Key words —— neurosteroid, sulfotransferase, brain, hydroxysteroid

INTRODUCTION

Sulfation of endogenous and xenobiotic compounds is an important conjugation reaction mediated by a family of enzymes known as sulfotransferases (SULTs).[1–3] Cytosolic SULTs catalyze the transfer of a sulfonyl group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to another compounds.

Neurosteroids such as dehydroepiandrosterone (DHEA) and pregnenolone, and their sulfated esters within the brain, are known to be potent regulators of neuronal activity.[4–7] They modulate the function of $GABA_A$ receptors[6–8] and NMDA receptors.[9,10] High amounts of DHEA sulfate and pregnenolone sulfate have been detected in the brains of castrated and adrenalectomized rats, suggesting the presence of SULT activity within the central nervous system.[11] Sulfation of 3β-hydroxysteroids is catalyzed by a specific SULT family, hydroxysteroid SULT (classified as SULT2), which is found in the liver among other tissues. Several SULT2 cDNAs have been cloned from mammalian livers, as well as other tissues. In humans, two types of SULT2 cDNAs have been isolated: SULT2A1 cDNA, expressed within the human liver and adrenal gland;[12,13] and SULT2B1, expressed within the human prostate and placenta.[14] The SULT2B1 gene encodes two SULT2B1 mRNAs, SULT2B1a and SULT2B1b, created by alternative splicing at the N-terminal region.[14] Although both enzymes have been observed to sulfonate DHEA and pregnenolone with similar efficacy,[15] little is known about their role in the synthesis of neurosteroid sulfates. Thus, in the present study, we characterized SULT2 expression within the human brain.

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MATERIALS AND METHODS

Materials —— A mixture of human normal whole brain cDNA (from a 58-year-old male and a 1-year-old female) was purchased from Biochain (Hayward CA, U.S.A.). A human hepatic cell line, HepG2, was obtained from RIKEN Cell Bank (Tokyo, Japan).

Cell Culture —— HepG2 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 10 units/ml penicillin and 10 units/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2.

RNA Extraction and RT-PCR —— Total RNA was isolated from the cultured HepG2 cells by guanidine thiocyanate phenol-chloroform extraction (Isogen, Nippon Gene Co. Ltd., Tokyo, Japan). The first strand of cDNA was synthesized from 10 μg of total RNA using 1 unit M-MLV reverse transcriptase

Table 1. Oligonucleotide Primers for PCR Amplifications

mRNA	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Size (bp)	Position
SULT2A1	ATGTCGGACGATTTCTTATG	ATTCCCATGGGAACAGCTC	856	21– 876
SULT2B1	GAAGTTGCCAGGTGAATAC	TTTATTATGAGGGTCGTGGG	1028	405–1432*
SULT2B1a	CGAGTGTCGCCACCCTGAGAACTC	AAGTCCCTCAGGAACTGGTCG	541	326– 866
SULT2B1b	GCTCCCTGCTCGTCCTCCCCTC	AAGTCCCTCAGGAACTGGTCG	578	40– 617
β-actin	GCCGTCTTCCCCTCCATCGT	TGTCACGCACGATTTCCCTC	550	126– 675

Nucleotide sequences were adopted from the Genbank. The accession IDs are as follows; SULT2A1(U08024), SULT2B1(U92314), SULT2B1a (U92314), SULT2B1b (U92315), β-actin (X00351). *The nucleotide numbers were adopted from the SULT2B1a cDNA sequence in the common region.

with oligo (dT) primers according to the manufacturer's protocol (Stratagene, Alameda, CA, U.S.A.). All PCR primers for the SULTs and β-actin were designed from the sequences published in GenBank. The primer sequences used are listed in Table 1. PCR amplification of SULT2A1, 2B1, 2B1a and 2B1b was performed with AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, U.S.A.) under following conditions: 9 min at 94°C, followed by 40 cycles at 94°C for 1 min, 58°C for 1.5 min, and 72°C for 2 min.

Cloning and Sequencing of Human Brain SULT2A1 and SULT2B1 cDNAs —— The SULT2A1 and SULT2B1 PCR amplification products from human brain cDNA were purified, ligated into pCR2.1 (Invitrogen, Carlsbad, CA, U.S.A.), and transformed into *E. coli* JM109. Plasmid DNA containing cDNA from either SULT was purified and sequenced. The sequence of SULT2B1b cDNA was determined directly from the PCR product.

Northern Blot Analysis —— Human Brain Multiple Tissue Northern Blot II and IV (Clontech, Polo Alto, CA, U.S.A.) were used to perform Northern blot analysis. Each lane of the Northern blot contained approximately 2 μg of poly $(A)^+$ RNA. The inserted SULT2A1 and SULT2B1 cDNAs were recovered with digestion by EcoRI and used as probes. The probes were labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham Pharmacia Biotech, Piscateway, NJ, U.S.A.) using a random prime labeling system (Rediprime™ II, Amersham Pharmacia Biotech). Each membrane was prehybridized for 2 hr and then hybridized for 18 hr with each probe at 68°C. The human glycerol-3-phosphate dehydrogenase (G3PDH) cDNA probe (Clontech) was hybridized as described above, except that a different hybridization temperature (65°C) was used to ensure RNA integrity and normalization of RNA loading. Radioactivity within each membrane was detected with BAS 2000 (Fuji Film, Tokyo, Japan) and quantified using Adobe Photoshop (Adobe, San Jose, CA, U.S.A.).

RESULTS

Identification of Hydroxysteroid SULT Expression in the Human Brain

Sulfation of 3-hydroxysteroids, such as DHEA and pregnenolone, is catalyzed by a specific SULT family, SULT2, found in the mammalian liver among other tissues. Judging from their substrate affinities, SULT2 enzymes are probably the most potent enzymes capable of catalyzing neurosteroid sulfation in the brain. In humans, two types of SULT2 cDNAs have been isolated: SULT2A1[12,13] and SULT2B1.[14] SULT2A1 is expressed in the human liver and adrenal gland, and SULT2B1 is expressed in the human prostate and placenta. However, little is known about their expression in the brain. In order to analyze their expression within the human brain, we measured SULT2 mRNA levels in the human brain by examining a mixture of human brain cDNA sequences by PCR. As shown in Fig. 1A, we detected both SULT2A1 and SULT2B1 within cDNA isolated from two human brains. The expression levels of both SULT2 mRNAs were very low compared to that of HepG2 SULT2A1. The SULT2B1 gene encodes two mRNAs, SULT2B1a and SULT2B1b, each having a different N-terminal amino acid sequence as a result of alternative splicing.[14] To distinguish between these two SULT2B1 transcripts, we performed isoform-specific PCR analysis and found that only SULT2B1b was expressed in the human brain (Fig. 1B).

Cloning of SULT2 cDNA from the Human Brain

To determine whether the SULT2 mRNAs expressed within the brain were identical to those found in other tissues, we cloned and sequenced their PCR

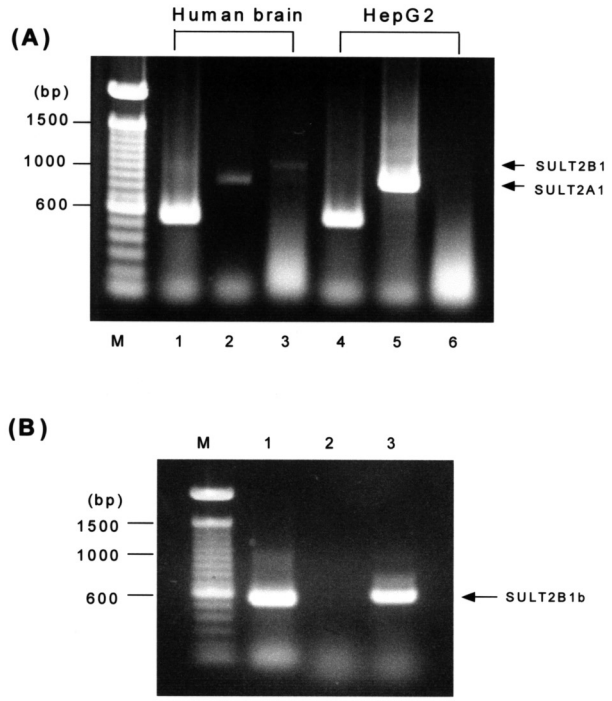

Fig. 1. Identification of SULT2A mRNAs in Human Brain by PCR

(A) Specific primers (Table 1) were used to amplify SULT2 cDNAs from the cDNA mixture of two human normal brains (lanes 1–3) and HepG2 cells (lanes 4–6). Lanes 1 and 4, β-actin; lanes 2 and 4, SULT2A1; lanes 3 and 6, SULT2B1; M, size markers. (B) Isozyme-specific PCR to detect SULT2B1a and SULT2B1b cDNAs in the human brains. Lane 1, b-actin; lane 2, SULT2B1a; lane 3, SULT2B1b; M, size markers.

products. The nucleotide sequence of brain SULT2A1 was identical to that of liver SULT2A1. However, three silent mutations (^{774}G→A, ^{915}C→T and ^{1029}T→C) were found in the nucleotide sequence of brain SULT2B1b when it was compared to the reported sequence.[14] We detected three mutations in two SULT2B1b clones and one mutation (^{915}C→T) in another. One of the two differing SULT2B1 genes might belong to each of the two individuals from whom the cDNA mixture was made.

Northern Blot Analysis of SULT2 Expression in the Human Brain

Expression of SULT2A1 and SULT2B1 in the human brain was investigated by Northern blot analysis of poly(A)$^+$ RNA isolated from 15 different brain regions. As shown in Fig. 2A, SULT2A1 mRNA was restricted to the thalamus/hypothalamus. The probe hybridized with three different-size poly(A)$^+$ RNAs. The two abundant messages are approx. 1900 and 1300 nucleotides in length, respectively. The sizes of the SULT2A1 message are almost similar to those of the human livers reported by Comer *et al.*[13]

On the other hand, SULT2B mRNA was detected throughout the brain (Fig. 2B). The size of the hybridized message was 1200–1300 nucleotides in length. This size is almost consistent with those of other human tissues reported by Her *et al.*[14] The relative levels of SULT2B1 expression could be determined by normalization with G3PDH levels, as shown in Table 2. The results indicate that SULT2B1 expression was two to three- fold higher in some regions of the brain than in others.

DISCUSSION

We detected expression of two types of SULT2, SULT2A1 and SULT2B1b, in the human brain, although very low levels of expression were detected, judging from the band intensities observed during PCR and Northern blot experiments (Figs. 1 and 2). These results are consistent with previous observations that 300 times less SULT activity occurs in rat brain homogenate than in rat liver homogenate.[16,17] It is not clear whether this is because only a small population of SULT-expressing cells exist in the brain, whether SULT is expressed only at very low levels in SULT-expressing cells within the brain, or both. However, immunohistochemical analysis suggests that expression of hydroxyl SULT enzymes is limited to specific cell types or specific cell regions within the brain.[17–19]

Surprisingly, SULT2A1 expression was only detected in the region of the thalamus and/or hypothalamus in the present experiment (Fig. 2). The physiological implication of this result is unclear, however, this finding seems to concur with the observation that DHEA SULT activity is highest within the thalamus of the frog brain.[17] Immunohistochemical analysis has revealed that the major site of neurosteroid biosynthesis is the mitochondria of oligodendrocytes.[20,21] The highest concentration of oligodendrocytes in the brain is found within the thalamus. It is interesting to speculate that oligodendrocytes in the thalamus might selectively express SULT2A1. The thalamus is an important part of the brain, responsible for distributing (and processing) most sensory and motor information to the cerebral cortex. Moreover, it regulates levels of awareness and emotional responses to sensory experiences. We suspect that SULT2A1 activity within the thalamus may play a crucial role in CNS functioning.

On the other hand, SULT2B1b transcripts were

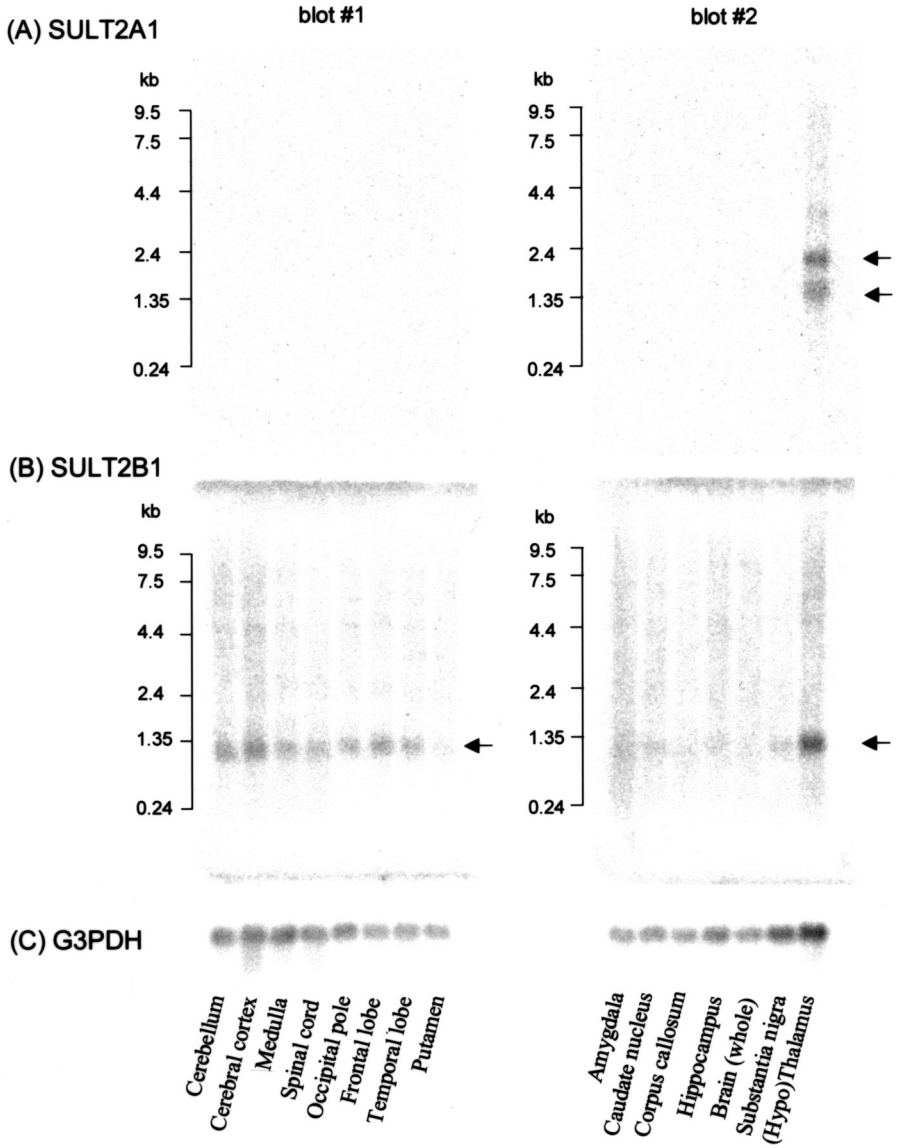

Fig. 2. Localization of SULT2A1 and SULT2B1b Expression in the Human Brain

Northern blots of mRNAs isolated from different parts of human brains were probed with two SULT2 cDNAs, respectively. Each lane contains approximately 2 μg of poly(A)$^+$ RNA from 15 different regions of human brains. Two blots (#1 and #2) were hybridized with ^{32}P-labeled SULT2A1 probe (A), SULT2B1 probe (B) or G3PDH probe (C). Hybridized radioactivity was visualized by BAS2000. Note that the exposure times were 16 hr for SULT2 and 2hr for G3PDH, respectively.

Table 2. Relative mRNA Levels of SULT2B1 in Individual Regions of Human Brain

Blot #1			Blot #2		
Regions	2B1/G3PDH	ratio	Regions	2B1/G3PDH	ratio
Cerebellum	0.71	(1.61)	Amygdala	0.24	(1.00)
Cerebral cortex	0.62	(1.41)	Caudate nucleus	0.30	(1.25)
Medulla	0.44	(1.00)	Corpus callosum	0.32	(1.33)
Spinal cord	0.48	(1.09)	Hippocampus	0.32	(1.33)
Occipital pole	0.82	(1.86)	Whole brain	0.26	(1.08)
Frontal lobe	1.19	(2.70)	Substantia	0.31	(1.29)
Temporal lobe	0.90	(2.05)	Thalamus/Hypothalamus	0.48	(2.00)
Putamen	0.53	(1.20)			

The ratio was calculated from the band intensities of SULT2B1 and G3PDH mRNAs in each slot. Parentheses indicate the relative ratios obtained by taking the minimum ratio of each blot as 1.00.

detected in all examined regions of the human brain, indicating expression of the SULT2B1 gene throughout the human brain (Fig. 1B). No SULT2B1a expression was detected: This observation agrees with recent data indicating that SULT2B1a expression is found only in the colon, ovary and fetal brain.[22,23] SULT2B1b expression was observed throughout the brain, with two to three- times greater expression noted in some areas than in others (Fig. 2B and Table 2). Among the regions examined, the frontal and temporal lobes, as well as the thalamus, were observed to express higher levels of SULT2B1b mRNA (Fig. 2). These results are consistent with the observation that expression of the cytochrome P450 side chain cleavage enzyme (P450scc), which produces pregnenolone from cholesterol in the mitochondria, is found throughout the brain, especially within the white matter.[21,24]

Our data imply differential regulation of expression of the two SULT2 genes in a cell-specific or region-specific manner in the human brain, although both SULT2 enzymes can sulfonate 3β-neurosteroids such as DHEA and pregnenolone. However, it is interesting to note that these enzymes have slightly different substrate preferences; SULT2A1 has a higher affinity for DHEA than pregnenolone, and SULT2B1b prefers pregnenolone to DHEA.[14,15] Further analysis of the individual activities of the SULT2A1 and SULT2B1b enzymes is required to define the physiological role of SULT2 isozymes within the brain.

We detected two sets of silent muations within SULT2B1 cDNA; three silent mutations (^{774}G→A, ^{915}C→T, ^{1029}T→C) were detected in two cDNA clones and one mutation (^{915}C→T) was detected in another. We presume that the one of the two differing SULT2B1 genes belong to each of the two individuals from whom the cDNA mixture was made. The silent mutation at the ^{915}C site has recently been identified as a single nucleotide polymorphism (SNP) that occurs in the Japanese population.[25] Since the other two mutations are not found in the Japanese population, they are likely SNPs unique to other races.

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