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Hydroxysteroid sulfotransferase 2B1b expression and localization in normal human brain

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Abstract

Steroid sulfonation in the human brain has not been well characterized. The major sulfotransferase (SULT) isoforms that conjugate steroids in humans are SULT1E1, SULT2A1, and SULT2B1b. SULT2B1b catalyzes the sulfonation of 3β-hydroxysteroids, including neurosteroids dehydroepiandrosterone and pregnenolone, as well as cholesterol and several hydroxycholesterols. SULT2B1b mRNA and protein expression were detected in adult and fetal human brain sections, whereas neither mRNA, nor protein expression were identified for SULT1E1 or SULT2A1. Using immunohistochemical analysis, SULT2B1b expression was detected in neurons and oligodendrocytes in adult brain and in epithelial tissues in 28-week-old fetal brain. Sulfonation of cholesterol, oxysterols, and neurosteroids in the brain is apparently catalyzed by SULT2B1b since expression of neither SULT2A1 nor SULT1E1 was detected in human brain sections. SULT2B1b mRNA and protein were also detected in human U373-MG glioblastoma cells. Both mRNA and protein expression of liver X receptor (LXR)-β, but not LXR-α, were detected in U373-MG cells, and LXR-β activation resulted in a decrease in SULT2B1b protein expression. Since hydroxycholesterols are important physiological LXR activators, this suggests a role for regulation of sterol metabolism by LXR and SULT2B1b. Therefore, elucidating key enzymes in the metabolism of cholesterol and neurosteroids could help define the properties of steroid conjugation in the human brain.

Keywords

brain; glioblastoma; human; liver X receptor; sulfotransferase; sulfotransferase (SULT) 2B1b

Introduction

Neurosteroids are a specific class of steroids synthesized in the brain that are necessary for normal brain development and function [1]. Dehydroepiandrosterone (DHEA) and pregnenolone (PREG) are two neurosteroids that contribute to brain development and are involved in the regulation of neurotransmission, synaptic plasticity, as well as memory and cognition [2]. DHEA and PREG also serve as precursors for steroid hormone biosynthesis, resulting in the formation of androgens and estrogens [3]. The biosynthetic pathway for neurosteroids is distinct from steroidogenesis in peripheral tissues, which involves adrenal,

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ovarian, and testicular hormones [1]. Multiple cell types, both neuronal and glial, are involved in neurosteroidogenesis [4]. Maintaining pools of these neurosteroids is a dynamic process and is important since neurosteroids are essential to normal brain development and function [5]. Synthesis and metabolism of neurosteroids in the brain are not well understood, but have been the subject of a recent investigation [6]. Elucidating the enzymes involved in the metabolism of DHEA and PREG is therefore necessary to more completely understand the role of neurosteroids in brain physiology.

Human cytosolic sulfotransferases (SULTs) are a family of phase II drug-metabolizing enzymes that catalyze the transfer of a sulfonate (SO_3^-) moiety from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an acceptor substrate with a hydroxyl or amino functional group [7]. The human SULTs are divided into two main families on the basis of substrate specificity. The SULT1 family conjugates small phenols, such as naphthols, estrogens, catecholamines, and dietary polyphenols, whereas the SULT2 family conjugates hydroxysteroids, such as steroid hormones, bile acids, and cholesterol [7]. The human SULT2 isoforms include SULT2A1 and SULT2B1b, and both isoforms conjugate hydro xysteroids [8]. The *SULT2B1b* gene encodes two transcripts that correspond to isoforms SULT2B1a and SULT2B1b [9]. SULT2B1a mRNA expression is detected in many tissues, but SULT2B1a protein expression has not been identified in humans [8, 10]. In contrast, SULT2B1b protein expression has been detected in the lung, skin, prostate, placenta, endometrium, and breast [10]. While there are limited reports of SULT2B1 isoform expression in the brain of rodents, rabbits, and zebrafish, there are no reports of SULT2B1b protein expression in the human brain [11], and reports concerning mRNA expression are conflicting [9, 12]. Our laboratory has previously reported that SULTs 1A1 and 1A3 are expressed in several human brain sections [13]. Substrates for SULT2B1b in the brain include DHEA, PREG, and cholesterol, which is the precursor for all steroid hormones [14, 15]. Oxysterols are also present in high concentrations in the brain and are predicted to be substrates for SULT2B1b [16, 17]. Our laboratory has recently reported the sulfonation of 24-hydroxycholesterol by SULT2B1b [18].

The human brain contains approximately 20%–30% of total body cholesterol, but accounts for only 2% total body mass [19]. This high concentration of cholesterol is necessary for maintaining the high rate of cellular metabolism, for the synthesis of myelin sheaths, and for enabling the salutatory conduction responsible for neurotransmission [20]. Brain cholesterol is synthesized de novo, and plasma cholesterol and dietary cholesterol intake have little influence on brain concentrations [21]. For this reason, it is predicted that brain cholesterol has a half-life of at least 5 years [22]. Not only is cholesterol synthesized de novo in the human brain, investigators have also shown that concentrations of DHEA, PREG, and their sulfate derivatives in the brain are distinct from plasma concentrations [19]. Since DHEA, PREG, and sterols are synthesized endogenously in the brain and appear to be tightly regulated, the brain SULT isoforms responsible for sulfonate conjugation of endogenous hydroxysteroids, sterols, and cholesterol should be elucidated.

Materials and methods

Materials

Oligonucleotide primers were synthesized by Operon (Huntsville, AL, USA). [^{35}S]-PAPS (2.2 Ci/mmol), [1,2,6,7-^{3} H]-DHEA (79 Ci/mmol), and [^{3}H]-PREG (25 Ci/mmol) were purchased from Perkin-Elmer (Boston, MA, USA). Non-radiolabeled PAPS was obtained from Dr. Sanford Singer (University of Dayton, Dayton, OH, USA). Affinity-purified goat anti-rabbit horseradish peroxidase (HRP) conjugate was purchased from Southern Biotechnology (Birmingham, AL, USA). Super Signal chemiluminescence substrate kit and Mammalian Protein Extraction Reagent (MPER) were purchased from Thermo Fisher

(Rockford, IL, USA). RNA STAT-60 was purchased from TelTest (Friendswood, TX, USA). Super Script Reverse Transcriptase II was obtained from Invitrogen (Carlsbad, CA, USA). RedTaq ReadyMix was purchased from Sigma (St. Louis, MO, USA). Random hexamer primers and dNTP mix were purchased from Promega (Madison, WI, USA). 3,3′-Diaminobenzidine (DAB) chromogen was purchased from BioGenex (San Ramon, CA, USA). Liver X receptor (LXR) agonist TO901317 was purchased from Cayman Chemical (Ann Arbor, MI, USA). U-373 MG glioblastoma cells were a generous gift from Dr. Karina Yoon [University of Alabama at Birmingham (UAB)]. MCF-7 cells were purchased from American Type Culture Collection (Bethesda, MD, USA). All other chemicals were reagent grade.

PCR amplification of SULT isoforms

Frozen brain sections were obtained from the Alzheimer's Disease Research Center (UAB). For isolation of total RNA, 50 mg tissue was homogenized in 750 μL of RNA STAT-60 (TelTest), and total RNA was isolated as described previously [23]. Total RNA was re-suspended in nuclease-free distilled water, and 2.5 μg was used in reverse transcription reactions for first-strand cDNA synthesis using Super Script Reverse Transcriptase II with random hexamer primers. Genes of interest were amplified using RedTaq ReadyMix with cDNA templates.

Immunoblot analysis

Cytosolic fractions were prepared from frozen adult brain sections, including the superior temporal gyrus, hippocampus, cerebellum, occipital lobe, frontal lobe, and temporal lobe by homogenizing 200 mg tissue in 1 mL phosphate buffer (10 mM $NaPO_4$, 150 mM NaCl, 10% glycerol, 1.5 mM DTT, pH 7.4). Homogenates were centrifuged at 100,000×*g* for 1 h at 4°C to obtain cytosol. For immunoblot analysis, cytosolic proteins were resolved by SDS-polyacrylamide gel electrophoresis and resolved proteins were transferred to nitrocellulose via semidry transfer. Membranes were then blocked in 5% non-fat milk in Tris-buffered saline (TBS) for 1 h before incubation with rabbit anti-SULT2B1b serum (1:1000) in 0.1% non-fat milk in TBS for 1 h. Immunoblots were then incubated in goat anti-rabbit HRP (1:50,000) in 0.1% non-fat milk for 1 h before developing in Super Signal West Pico (Thermo Fisher, Rockford, IL, USA) and exposure to an autoradiographic film. Bacterially expressed, purified recombinant human SULT2B1b was used as a control in these experiments. A specific SULT2B1b polyclonal antibody was generated by our laboratory [8]. Briefly, 90 μg purified recombinant 6His-SULT2B1b was mixed with Freund's complete adjuvant before subcutaneous injection along the back of a New Zealand white rabbit. A booster injection was given after 2 weeks with 90 μg purified recombinant 6His-SULT2B1b that was mixed with Freund's incomplete adjuvant. After an additional 2 weeks, serum from the rabbit was tested in immunoblot analysis to evaluate the serum for polyclonal antibodies against SULT2B1, and to validate the specificity of the anti-serum.

Immunohistochemical analysis

Formalin-fixed paraffin-embedded human brain sections were obtained from the Tissue Procurement Center of the UAB Comprehensive Cancer Center. Serial 5 μm sections were cut from the paraffin blocks, mounted to poly-lysine-coated glass slides, and oven dried. Slides were deparaffinized and rehydrated in xylenes and alcohol baths (absolute, 95%, 75%), then rinsed in Tris bath (50 mM Tris, 150 mM NaCl, 0.01% Triton X-100, pH 7.6), blocked in 1% goat serum for 1 h, and incubated with SULT2B1b anti-serum (1:100) in PBE [phosphate-buffered saline (PBS), 1 mM EDTA, 1% bovine serum albumin, pH 7.6] for 1 h. No antigen retrieval techniques were required for optimal immunoreactivity. Slides were washed twice in Tris bath, and incubated in conjugated biotin (1:500) in PBE for 10

min, then incubated in streptavidin-HRP (1:500) in PBE for 5 min before staining with DAB and counterstaining with hematoxylin. Slides were rinsed in water before dehydrating by incubating in 70%, 95%, and 100% ethanol, then xylenes for 2 min each before coverslip mounting. Photomicrographs were taken using a Zeiss AxioStar microscope fitted with camera and AxioVision software (Dublin, CA).

Immunoblot analysis of U373-MG cells

U373-MG cells and MCF-7 breast cancer cells were grown to confluency in 10 cm^2 dishes in DMEM/10% FBS. Cell lysates were prepared using MPER and then cytosol was prepared by centrifugation at 100,000×*g* for 1 h at 4°C. For immunoblot analysis, 100 μg protein was resolved in 10% SDS-polyacrylamide gels. Resolved proteins were transferred to nitrocellulose using semidry transfer and immunoblotted as described above with either SULT2B1b anti-serum (1:1000) or anti-LXR IgG (1:2500).

DHEA sulfonation in U373-MG cells

U373-MG cells were seeded into 12-well plates and allowed to grow to confluency in DMEM/10% FBS. Cells were washed three times with PBS, then incubated in steroid-free medium for 24 h before the addition of [^{3}H]-DHEA (20 μM) to the medium. Aliquots of the culture medium were assayed for the presence of [^{3}H]-DHEA-sulfate ([^{3}H]-DHEA-S) at 3, 6, 12, and 22 h. Scintillation spectroscopy was utilized to quantify total [^{3}H]-DHEA-S after removal of non-sulfated [^{3}H]-DHEA by $CHCl_3$ extraction [24].

Treatment of U373-MG cells with T0901317

U373-MG cells were seeded into six-well plates at 0.5×10^6 cells/well. After 24 h, the medium was removed, and the cells were washed with PBS and placed in steroid-free medium. Cells were incubated in medium containing 0, 2.5, and 5 μM TO901317 for 48 h. After treatment, cell lysates were prepared using MPER. Cytosol was prepared from cell lysates by centrifugation at 100,000×*g*. Cytosol from control and TO901317-treated cells was immunoblotted as described above.

Results

PCR amplification of SULT isoforms

PCR was initially used to identify the expression of SULT2B1b in several different regions of the adult human brain. Total RNA was isolated from specimens of prefrontal cortex, hippocampus, and cerebellum of normal human brains and used in the specific amplification of SULT2B1b mRNA. PCR amplification of total RNA showed SULT2B1b mRNA expression in all sections of brain analyzed (Figure 1). Consistent with previous reports, SUT2A1 and SULT1E1 (data not shown) mRNA expression were not detected [8].

Immunoblot analysis of SULT2B1b in human brain sections

Since SULT2B1b mRNA expression was detected in the brain, expression of SULT2B1b protein was evaluated by immunoblot analysis using cytosol prepared from several brain regions: superior temporal gyrus, hippocampus, cerebellum, occipital lobe, frontal lobe, and temporal lobe. Readily detectable levels of SULT2B1b immunoreactive protein were found in each section of brain analyzed (Figure 2). Since the rabbit SULT2B1b antibody was raised against purified full-length recombinant SULT2B1b, it reacts with both SULT2B1a and SULT2B1b [8]. Because the N-terminal end of SULT2B1a is 16-amino-acid shorter than SULT2B1b, the two isoforms are readily identifiable by immunoblot analysis [8]. As observed in peripheral tissues, no SULT2B1a immunoreactive protein was observed in the

brain specimens. Also, neither SULT1E1 nor SULT2A1 protein expression was detected in the same brain sections (data not shown).

Immunohistochemical analysis of SULT2B1b in human adult and fetal brain sections

To determine the brain cell types that express SULT2B1b, formalin-fixed adult brain sections were immunostained with rabbit anti-SULT2B1b b serum (Figure 3). Immunoreactive SULT2B1b protein was found in all tissue sections analyzed, and expression appeared to be located in neurons and oligodendrocytes. Oligodendrocytes are histologically characterized by their round nuclei and perinuclear halo [25]. The colocalization of DAB staining in the cytosol of cells with this morphology supports the expression of SULT2B1b in oligodendrocytes. The cells did not show immunostaining for glial acid fibrillary protein (data not shown), which is not expressed in mature oligodendrocytes [26]. Neuronal expression of SULT2B1b is identifiable by the DAB staining in pyramidal-shaped neurons and colocalization of the neuronal-specific antibody NeuN (data not shown). As shown in Figure 3, SULT2B1b immunoreactivity was not apparent in all neurons or all oligodendrocytes.

Unlike other phase II drug-metabolizing enzymes, several of the SULT isoforms are readily expressed in the developing human fetus [27]. To determine whether SULT2B1b is present in the fetal brain, expression of SULT2B1b during late fetal development (40 weeks' gestational age) was evaluated by immunohistochemical analysis. Figure 4 shows that SULT2B1b protein was detected in both the hippocampal and frontal cortex regions. Immunoreactivity appears associated with neurons in the hippocampal section, and with both neurons and glial cells in the frontal lobe section.

In contrast to the SULT2B1b staining in the 40-week brain, little immunoreactivity was observed in sections of the 28-week-old fetal brain (Figure 5). However, SULT2B1b staining was observed in developing hair follicles in epithelial tissue included in the sections (Figure 5A–D). Also, immunoreactivity was observed in primitive cartilage with perichondrium (Figure 5E and F) in sections from the base of the skull.

Immunoblot analysis in U373-MG cells

To better investigate the regulation and role of SULT2B1b expression in the brain, several human brain cell lines were evaluated for SULT2B1b expression. U373-MG glioblastoma cells, SH-SY5Y neuroblastoma cells, and CCF-STTG1 astrocytoma cells were grown to confluency in 100-cm dishes; cytosol was prepared and then immunoblotted using rabbit anti-SULT2B1b sera. No SULT2B1b expression was detected in CCF-STTG1 astrocytoma cells or SH-SY5Y neuroblastoma cells by PCR or immunoblot analysis (data not shown). SULT2B1b expression was readily detectable in U373-MG cytosol by immunoblot analysis (Figure 6). Similar to the brain studies, SULT2B1a was not detected in the U373-MG cells. SULT2B1b immunoreactive protein in U373-MG cells was comparable to levels of SULT2B1b immunoreactive protein in MCF-7 epithelial breast tumor cells, which were used as a positive control since the mRNA and protein expression of SULT2B1b in MCF-7 cells has been well characterized [28]. Neither SULT2A1 nor SULT1E1 were detectable in the brain cancer cell lines by PCR or immunoblot analysis (data not shown).

DHEA sulfonation in U373-MG cells

Unlike other SULT isoforms, SULT2B1b activity cannot be detected in tissue lysates or homogenates [28]. While SULT2B1b activity has been detected in intact cells and nuclei, lysis of the cells or nuclei results in a rapid loss of sulfonation activity [28]. To further validate the expression of SULT2B1b in U373-MG cells, sulfonation activity was assayed in intact cells [28]. Figure 7 shows DHEA sulfonation activity in the medium of cultured intact

U373-MG cells. DHEA sulfonation was identified at 12 h and continued to increase over 22 h (Figure 7). [^{3}H]-DHEA-S was not detected within U373-MG cells (data not shown), suggesting that newly synthesized [^{3}H]-DHEA-S was readily excreted into the medium via organic anion transport systems.

LXR expression in U373-MG cells

Oxysterols, such as 24-hydroxycholesterol, are substrates for SULT2B1b as well as important endogenous ligands for LXR [18]. While reports in human tissues are limited, it appears that 24-hydroxycholesterol is only formed within the brain, and not in peripheral tissues [22]. The conversion of cholesterol to 24-hydroxycholesterol is thought to be a clearance mechanism for the transport of excess cholesterol from the brain [29]. Additionally, LXR activation has been shown to regulate SULT2B1b mRNA expression in several cell types, including aortic endothelial cells, keratinocytes, macrophages, and murine dendritic cells [30, 31]. Since activation of LXR and transcriptional activity of LXR response elements could affect cholesterol and steroid metabolism in the brain [32], the expression of LXR and the influence of LXR activation on SULT2B1b expression in U373-MG cells were explored. LXR-β, but not LXR-α, mRNA expression was detected by PCR analysis in total RNA isolated from U373-MG cells (Figure 8A). Similarly, immunoreactive LXR-β was detected in U373-MG cytosol, whereas LXR-α protein expression was not observed (Figure 8B).

Since LXR-β is expressed in U373-MG glioblastoma cells, and SULT2B1b has been implicated in the regulation of LXR-mediated lipid metabolism [33], the effect of LXR agonist TO901317 on SULT2B1b expression was investigated. U373-MG cells were treated for 48 h with TO901317 then cytosol was prepared for immunoblot analysis. As shown in Figure 9, SULT2B1b protein expression in the U373-MG cells decreases following treatment with increasing concentrations of TO901317.

Conclusions

SULT1E1, SULT2A1, and SULT2B1b are the major SULT isoforms that catalyze the sulfonation of steroids, such as estrogens, androgens, and PREG [34]. The expression of SULT isoforms in the human brain has not been well characterized. Consistent with previous reports, SULT1E1 and SULT2A1 expression has not been reliably reported in the human brain [8, 14]. Neither mRNA nor protein expression of SULT2A1 in the human brain has been reported. To date, only mRNA expression of SULT2B1b in the brain has been reported, and these reports are contradictory. Kohjitani et al. [35] report that SULT2B1a, but not SULT2B1b, is predominately found in the human brain. No mRNA expression of SULT2B1b was detected in Northern blot analysis by Her et al. [9]. In contrast, Shimizu [12] described an abundance of SULT2B1b mRNA in several sections of the human brain, which is consistent with the current report. Not only are SULT2B1a and SULT2B1b mRNA detected in the human brain, but SULT2B1b protein expression has also been identified for the first time in several fetal and adult brain specimens. Although SULT2B1a mRNA was detected, no SULT2B1a immunoreactive protein was observed, which is consistent with reports of a lack of detectable SULT2B1a protein expression in peripheral tissues [8].

SULT2B1b expression was localized primarily in neurons and oligodendrocytes in adult brain sections, and within the cells immunoreactive SULT2B1b protein localization appears to be confined to the cytosol. SULT2B1b is the only SULT isoform to show nuclear translocation in some cell types, primarily placental and breast [28, 36]. Human breast and breast tumor show varied ratios of cytosolic to nuclear localization of SULT2B1b, while SULT2B1b is detected almost entirely in the nuclei in term placenta [28, 36]. In contrast, human lung, prostate, and brain appear to express only cytosolic SULT2B1b [28, 37].

Nuclear localization in BeWo choriocarcinoma cells is regulated in part by phosphorylation of the unique C-terminal peptide of SULT2B1b [38]; however, phosphorylation of SUTL2B1b in the brain remains to be established.

There may be many substrates for SULT2B1b in the human brain since SULT2B1b catalyzes the conjugation of hydroxysteroids and neurosteroids [20]. The biosynthetic pathway for neurosteroids requires multiple brain cell types as enzymes necessary for the conversion of steroids from their precursors and the transport proteins, such as 3-β-hydroxysteroid dehydrogenase, required for their export and import are differentially expressed [39]. The consequences of the sulfonation of neurosteroid and hydroxycholesterols in the brain appear distinct from steroid metabolism in peripheral tissues. The sulfonation of DHEA, PREG, cholesterol, and oxysterols has been shown to confer unique functions to these molecules that the unconjugated forms do not possess [40]. DHEA-S and PREG sulfate (PREG-S) act as non-competitive $GABA_A$ antagonists [41]. PREG-S also interacts with *N*-methyl-D-aspartic acid receptors as a positive allosteric modulator [42]. It is therefore important to explore the mechanism by which neurosteroids DHEA-S and PREG-S are conjugated since these sulfated species have many important functions in the brain.

LXR is an important transcription factor for normal brain physiology as it regulates cholesterol, glucose, and fatty acid homeostasis [43]. Oxysterols are endogenous ligands for LXR, have been reported to be substrates for SULT2B1b [17, 18], and are present in high concentrations in the brain [16]. The sulfated oxysterol 24*S*-hydroxycholesteol-3-sulfate was recently shown to inhibit LXR-α activation by blocking coactivator recruitment [18]. The consequences of SULT2B1b-mediated inhibition of LXR activation were demonstrated by Villablanca et al. [44] as overexpression of SULT2B1b in murine dendritic cells regulates tumor growth. Additionally, Bai et al. [30] demonstrated that 25-hydoxyc-holesterol sulfate prevented LXR activation in human aortic endothelial cells, which led to decreased cellular lipid levels. In human cultured keratinocytes, peroxisome proliferator-activator receptor and LXR activation significantly increased SULT2B1b mRNA expression [31]. In contrast, in U373-MG cells, activation of LXR signaling by agonist TO901317 decreases the protein expression of SULT2B1b (Figure 9). LXR-mediated lipid metabolism in the brain, particularly regarding the role of LXR in 24-hydroxycholesterol excretion, may be regulated by SULT2B1b in a negative-feedback pathway. More specifically, the role of SULT2B1b in LXR signaling is twofold. The sulfated ligands for LXR can inhibit the activation of LXR, while the metabolism of these activators also reduces the available pool of LXR activators, such as oxysterols.

Metabolism and conjugation of cholesterol and oxysterols are major mechanisms for cholesterol recycling in the brain. Understanding metabolic pathways for cholesterol, oxysterol, and neurosteroids synthesis and metabolism is therefore important to elucidate their functions in the brain. Since SULT2B1b is the major hydroxysteroid SULT isoform expressed in the brain, characterization of the role of SULT2B1b is needed for a more complete understanding of cholesterol, oxysterol, and steroid metabolism in the brain.

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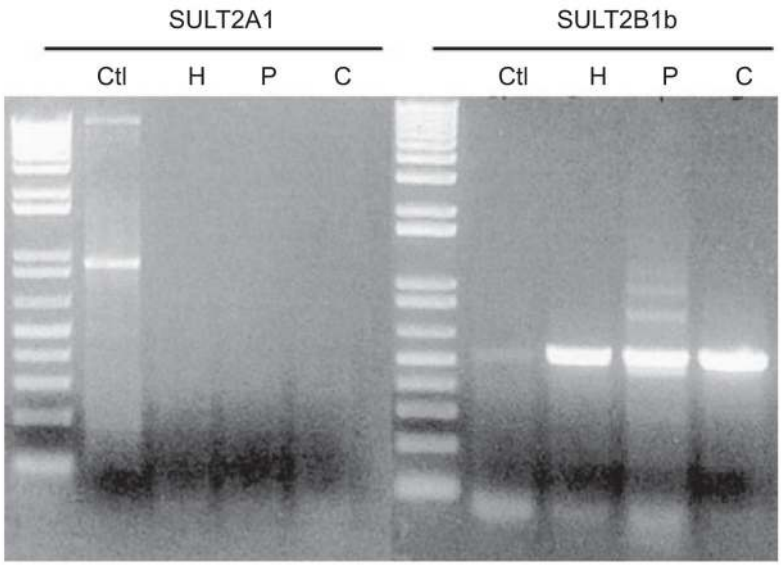

Figure 1.

SULT2B1b mRNA expression in isolated human brain sections. Total RNA was isolated from human brain sections – pre-frontal cortex (P), hippocampus (H), and cerebellum (C) – and cDNA first-strand synthesis was performed using Super Script Reverse Transcriptase II. The cycling parameters for PCR amplification included an initial denaturation 94°C for 2 min, then 29 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, followed by a final extension at 72°C for 5 min. Amplicons from PCR analysis were 821 bp for SULT2A1 (A) and 607 bp for SULT2B1b (B). Control lane expression is liver for SULT2A1 (A) and pcDNA3.1/SULT2B1b expression vector diluted 1:10 for SULT2B1b (B).

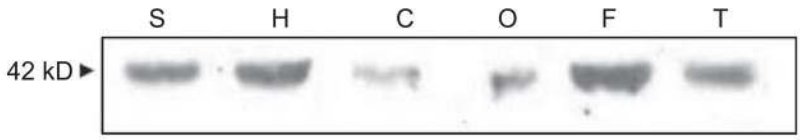

Figure 2.

SULT2B1b immunoreactivity in normal human adult brain sections. Cytosol (100 μg) was prepared from normal human brain sections and then electrophoresed, transferred to nitrocellulose, and blocked in 5% non-fat milk/TBS for 1 h. Immunoblots were incubated in rabbit anti-SULT2B1b serum (1:1000) in 0.1% milk for 1 h and then in goat anti-rabbit-HRP (1:50,000) for 1 h. Immunoblots were developed in Super Signal West Pico and exposed to an autoradiographic film. Brain sections shown: superior temporal gyrus (S), hippocampus (H), cerebellum (C), occipital lobe (O), frontal lobe (F), and temporal lobe (T).

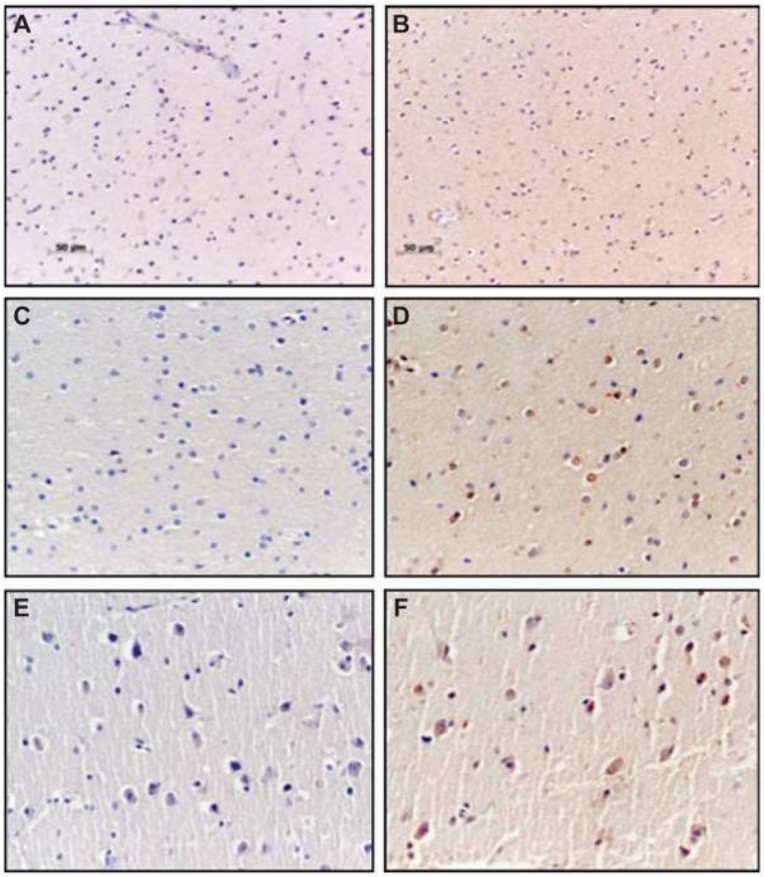

Figure 3.
Immunohistochemical analysis of SULT2B1b in human brain sections. SULT2B1b immunoreactive cells were detected in human pituitary (A, B), hippocampus (C, D), and frontal lobe (E, F). Slides were prepared from formalin-fixed human brain and were blocked in 1% goat serum, then incubated for 1 h with anti-SULT2B1b serum (1:100) in PBE (B, D, F). Control slides (A, C, E) were incubated in goat serum only. Slides were counterstained in hematoxylin after biotin-streptavidin labeling with DAB. Images shown at 20× (A–D) and 40×(E–F) magnification.

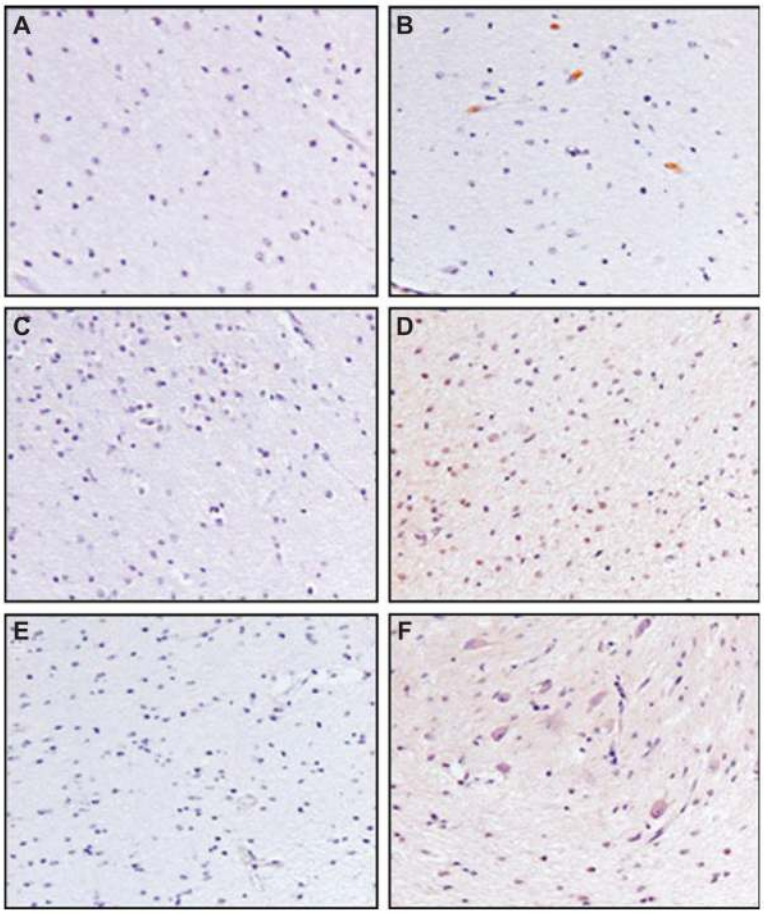

Figure 4.

Immunohistochemical analysis of SULT2B1b expression in human fetal brain at 40 weeks' gestation. Slides were prepared from formalin-fixed brain sections by the UAB Comparative Anatomy Laboratory and were blocked in 1% goat serum then incubated for 1 h with rabbit anti-SULT2B1 antisera (B, D, F) in PBE diluted 1:100. Control slides (A, C, E) were incubated in goat serum only. Fetal hippocampus is shown in panels A and B, and frontal lobe is shown at two different magnifications in panels C–F. Slides were counterstained in hematoxylin after streptavidin labeling with DAB. Images shown are 20× (panels A–D) and 40×(panels E and F) magnification.

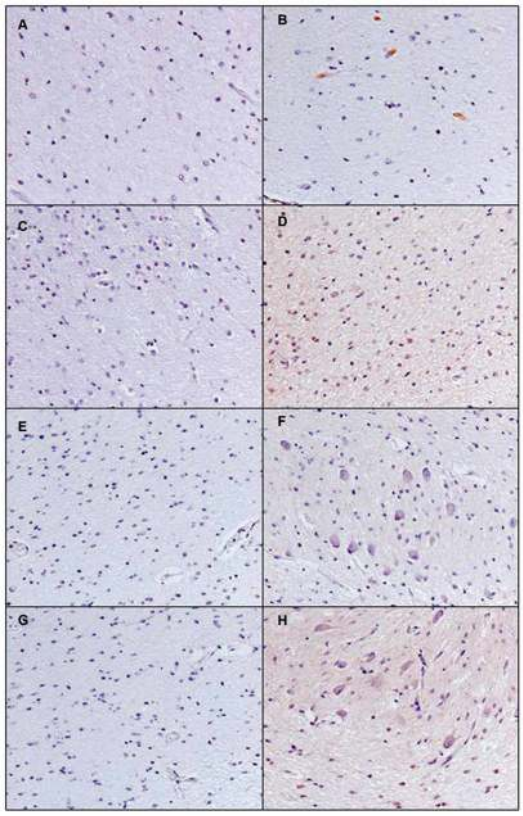

Figure 5.
Immunohistochemical analysis of human fetal brain at 40 weeks gestation. Slides were prepared from formalin-fixed brain sections by the UAB Comparative Anatomy Laboratory and were blocked in 1% goat serum and incubated for 1 h with anti-SULT2B1-body (B, D, F, H) in PBE diluted 1:100. Control slides (A, C, E, G) were incubated in goat serum only. Fetal hippocampus is shown in slides A, B; and frontal lobe is shown at two different magnifications in C–H. Slides were counterstained in hematoxylin after streptavidin labeling with DAB. Images shown are 20× and 40× magnification.

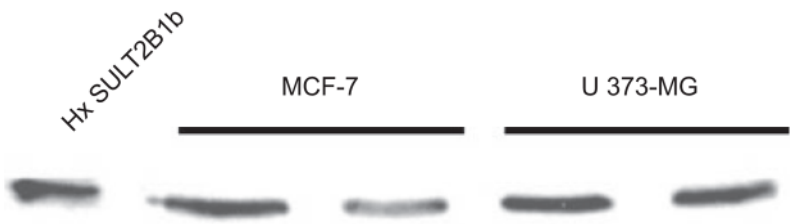

Figure 6.

Immunoblot analysis of SULT2B1b in U373-MG cells. Cytosol from confluent U373-MG cells and MCF-7 (100 μg) was electrophoresed, resolved proteins transferred to nitrocellulose, and blocked in 5% non-fat milk/TBS for 1 h. Immunoblots were incubated in rabbit anti-SULT2B1b serum (1:1000) in 0.1% milk for 1 h and then in goat anti-rabbit-HRP (1:50,000) for 1 h. Super Signal West Pico was used to develop immunoblots before exposure to autoradiographic film. Purified recombinant SULT2B1b, along with the MCF-7 cell cytosol were used as positive controls. Each cell type is shown in duplicate.

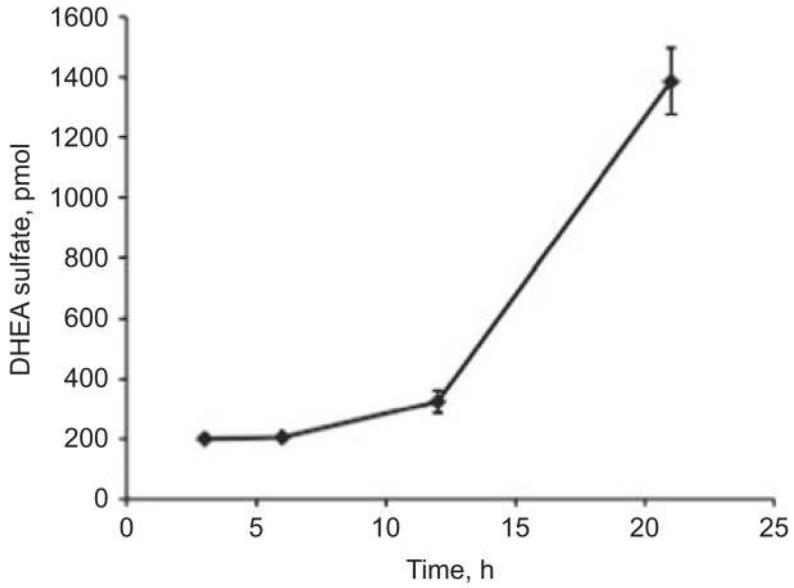

Figure 7.

DHEA sulfonation activity in U373-MG cells. Confluent monolayers of U373-MG cells were incubated in medium containing [^{3}H]-DHEA. The medium was assayed at 3, 6, 12, and 22 h for sulfonation activity by chloroform extraction and scintillation spectroscopy. Extraction blanks were used by extracting medium from wells containing no cells as well as wells containing cells at time zero. Error bars represent SEM, n ≥4.

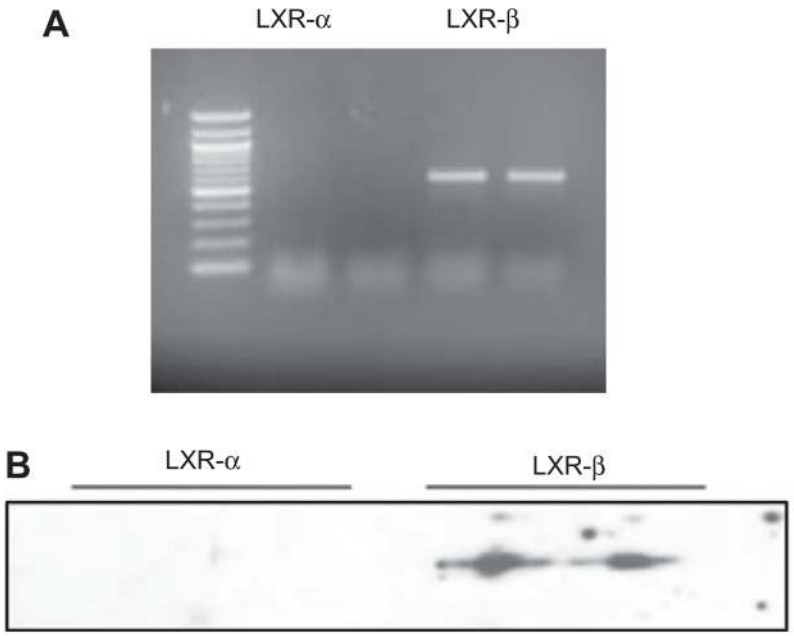

Figure 8.

Expression of LXR in U373-MG human glioblastoma cells. PCR analysis (A) of total RNA isolated from U373-MG cells. Gene-specific primers for LXR-α and LXR-β were used. The cycling parameters for PCR amplification included an initial denaturation 94°C for 2 min, then 29 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 2 min, followed by a final extension at 72°C for 5 min. For B, cytosol from confluent U373-MG cells was immunoblotted with rabbit anti-LXR-α IgG or anti-LXR-α/β IgG (1:1000) in 0.1% milk for 1 h and then in goat anti-rabbit-HRP (1:50,000) for 1 h. Super Signal West Pico was used to develop the immunoblot before exposure to an autoradiograph film. Each isoform type is shown in duplicate lanes.

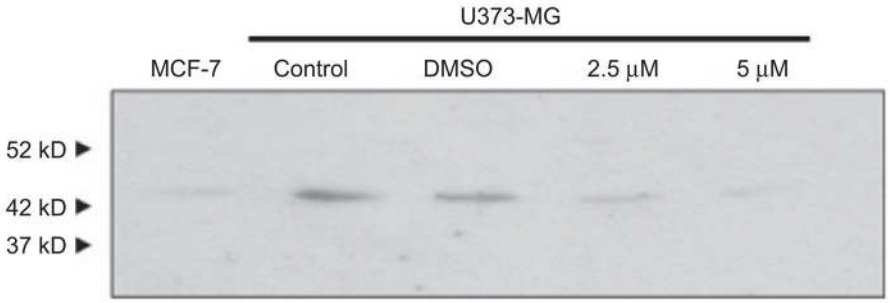

Figure 9.

Inhibition of SULT2B1b expression in U373-MG cells by treatment with LXR agonist TO901317. U373-MG cells were incubated in steroid-free medium containing 0, 2.5, and 5 μM TO901317 in DMSO for 48 h. TO901317-treated cells were lysed in MPER, then cytosol was prepared. Total cytosolic proteins were electrophoresed, and resolved proteins were transferred to nitrocellulose and blocked in 5% non-fat milk/TBS for 1 h. Blots were incubated in rabbit anti-SULT2B1b serum (1:1000) in 0.1% milk for 1 h and then in goat anti-rabbit-HRP (1:50,000) for 1 h. Super Signal West Pico was used to develop the immunoblots before exposure to an autoradiographic film. MCF-7 cell cytosol was used as a positive control.