Effects of Phenobarbital on Expression of UDP-Glucuronosyltransferase 1a6 and 1a7 in Rat Brain

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ABSTRACT

UDP-glucuronosyltransferase (UGT), a phase II drug-metabolizing enzyme, is expressed in the brain and can catalyze glucuronidation of endogenous and exogenous substances in the brain. Thus, changes in UGT1A expression could affect homeostasis and drug efficacy. Phenobarbital (PB), a typical inducer of drug-metabolizing enzymes, has been reported to induce oxidative stress and epigenetic changes, which could alter UGT expression in the brain. Here, we aimed to clarify the effects of PB on Ugt1a6 and Ugt1a7 gene expression in rat brains. Sprague-Dawley rats were treated intraperitoneally with PB (80 mg/kg), once daily for 7 days. Ugt1a6 and Ugt1a7 mRNA expression levels were increased in the striatum and thalamus (Ugt1a6, 3.0- and 2.9-fold, respectively; Ugt1a7, 2.6- and 2.6-fold, respectively). Acetaminophen glucuronidation was also increased in the medulla oblongata and thalamus by 1.8- and 1.2-fold, respectively. The induction rates within different brain regions were correlated with Ugt1a6 and Ugt1a7 mRNA expression, and the degree of induction also correlated with that of NF-E2–related factor-2 mRNA. Measurement of oxidative stress markers suggested that PB induced oxidative stress in brain regions in which Ugt1a6 and Ugt1a7 mRNAs were increased. Moreover, histone modifications were altered by PB treatment, resulting in increased histone H3 lysine 4 trimethylation in the striatum and thalamus and decreased histone H3 lysine 9 trimethylation in the thalamus. These results suggested that oxidative stress and histone modifications may promote transcriptional activation of Ugt1a6 and Ugt1a7 genes. In summary, Ugt1a6 and Ugt1a7 mRNA levels were increased by PB treatment, which may alter pharmacokinetics in the brain.

Introduction

Many drug-metabolizing enzymes (DMEs) are expressed primarily in the liver and play important roles in the metabolism of various exogenous and endogenous compounds. Because some DMEs are also found in extrahepatic tissues, including the brain, local brain metabolism could affect the efficacy of central-acting drugs and endogenous compounds, independent of liver metabolism (Dutheil et al., 2008; Mikys and Tyndale, 2013). UDP-glucuronosyltransferase (UGT) catalyzes a major phase II reaction and has been reported to be expressed in brain tissues, neurons, and astrocytes (Suleman et al., 1998; King et al., 1999; Shelby et al., 2003). Therefore, UGTA1s in the brain would be involved in mediating the pharmacological effects of several central-acting drugs as UGT substrates, such as acetaminophen (APAP) (Court et al., 2001), amitriptyline (Green et al., 1998), and valproic acid (Ethell et al., 1998). UGT1A also catalyzes the glucuronidation of neurotransmitters and neurosteroids, such as serotonin (Krishnaswamy et al., 2003), dopamine (Itäaho et al., 2009), and estradiol (Court, 2005), as endogenous substrates in the brain. Thus, changes in the expression levels of UGT1As in the brain may affect drug efficacy and homeostasis.

The expression levels of UGT are regulated by various inducers through nuclear receptors, such as aryl hydrocarbon receptor (Münzel et al., 2003; Yueh et al., 2005), constitutive androstane receptor (CAR) (Sugatani et al., 2005), and NF-E2–related factor-2 (Nrf2) (Shen and Kong, 2009). Phenobarbital (PB), a major antiepileptic drug, is a typical DME inducer functioning through CAR signaling pathway and activation of target gene transcription. Ugt1a6 mRNA has been reported to be increased by PB in the rat liver (Kato et al., 2005). Moreover, previous studies have revealed that PB induces oxidative stress in the rat liver and brain (Dostalek et al., 2008; Reeta et al., 2011). Oxidative stress can activate the Nrf2 signaling pathway, and Ugt1a6 and Ugt1a7 mRNA levels are increased by oxidative stress in rat astrocytes (Reeta et al., 2011). Since Ugt1a6 is expressed in the rat brain, neurons, and astrocytes (Shelby et al., 2003; Suleman et al., 1998), PB is thought to have the potential to change the expression of Ugt1a6 and Ugt1a7 isoforms in the rat brain.

PB can also alter DNA methylation and histone modifications (Watson and Goodman, 2002; Lempiäinen et al., 2011). Such epigenetic changes are involved in gene regulation prior to transcriptional activation. In a previous study, PB-mediated changes in DNA methylation and histone modification were shown to be associated with the upregulation of cytochrome P450 2b10 (a CAR target gene) in mice (Lempiäinen et al., 2011). Moreover, CAR activation has been shown to be related to epigenetic changes at the promoter region of CAR target genes (Chen et al., 2003; Yueh et al., 2005). Thus, Ugt1a6 and Ugt1a7 can be induced by PB and affect drug metabolism and homeostasis in the brain.

The purpose of this study was to ascertain whether changes in Ugt1a6 and Ugt1a7 occurred in rat brain because of PB. If such changes occurred, we sought to clarify their mediating mechanisms.

ABBREVIATIONS: APAP, acetaminophen; CAR, constitutive androstane receptor; DME, drug-metabolizing enzyme; H3K4, histone H3 lysine 4; H3K9, histone H3 lysine 9; NAC, N-acetyl-L-cysteine; Nrf2, NF-E2–related factor-2; PB, phenobarbital; PCR, polymerase chain reaction; TBARS, thiobarbituric acid–reactive substances; UGT, UDP-glucuronosyltransferase.
Phenobarbital Alters Brain UGT in Rats

**Materials and Methods**

Materials. Thiobarbituric acid was purchased from Cayman Chemical Company (Ann Arbor, MI). N-Acetyl-L-cysteine (NAC), APAP, barbital, PB, and 1,1,3,3-tetramethoxypropan were purchased from Wako Pure Chemicals (Osaka, Japan). APAP glucuronide was obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest grade commercially available.

Animal Treatment. The present study was approved by the Institutional Animal Care and Use Committee of Meijo University. Eight-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were treated intraperitoneally once daily for 7 days with PB at a dose of 80 mg/kg per day or with saline as the control. Twenty-four hours after the last treatment, the cerebellum, frontal cortex, parietal cortex, piriform cortex, hippocampus, medulla oblongata, olfactory bulb, striatum, and thalamus were excised from the brain.

RNA Extraction and Quantitative Real-time Polymerase Chain Reaction. Total RNA was extracted using TRIzol reagent (Life Technologies/ThermoFisher Scientific, Waltham, MA) and was then treated with DNase I (Promega, Madison, WI). Reverse transcription was conducted with ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan) according to the manufacturer’s instructions. The cDNA was synthesized by Greiner Bio-one (Tokyo, Japan) with SYBR Premix Ex Taq II (TaKaRa Bio). The PCR amplification conditions were as follows: initial denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 5 s; and annealing and extension at 60°C for 30 s. Primer sequences prepared for the Ugt standard solution and those used for quantification of UGT mRNA are described in Table 1 and synthesized by Greiner Bio-one (Tokyo, Japan). Using the average molecular weight of the amplified product and Avogadro’s constant, the number of copies per unit volume was calculated. The standard series and samples were run under the same conditions. PCR was performed using Thermal Cycler Dice instrument from TaKaRa Bio (Shiga, Japan) with SYBR Premix Ex Taq II (TaKaRa Bio). The primers used for quantification of UGT mRNA are shown as the mean values from five rats in each group.

Preparation of Microsomes. Pooled rat brain microsomes were prepared from 10 rats according to the methods described previously (Sakakibara et al., 2015) and stored at –80°C until analysis. Protein concentrations were determined using Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA).

APAP Glucuronidation. APAP glucuronidation was determined using the method described by Sakakibara et al. (2014) with slight modifications. In a preliminary study, we confirmed the linearity of the protein concentrations, substrate concentration, and incubation times. The concentrations of APAP and brain microsomes were 1 mM and 0.5 mg/ml, respectively. The reaction mixture was incubated at 37°C for 60 minutes. Data are presented as the mean of three independent determinations using pooled brain microsomes from 10 rats.

Immunoblot Analysis. The pooled cytoplasmic and nuclear extracts of the striatum and thalami from rat brains and livers were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (ThermoFisher Scientific) according to the manufacturer’s instructions. The cytoplasmic and nuclear extracts were collected and stored at –80°C until SDS-PAGE and immunoblotting. Immunodetection was performed with a polyclonal rabbit anti-CAR antibody (ab35850; abcam, Cambridge, MA) at 1:1000 dilution. Immunodetection for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Lamin B1 was carried out using anti-GAPDH antibody (ab8245; 1:5000 dilution; abcam) and anti-Lamin B1 antibody (ab16048; 1:5000 dilution; abcam), respectively. The bands were scanned using ImageQuant LAS-4010 instrument and quantitated with ImageQuant TL (GE Healthcare Life Sciences, Uppsala, Sweden).

Thiobarbituric Acid Reactive Substances Measurement. For determination of thiobarbituric acid–reactive substances (TBARS) levels, brain tissues from five rats were pooled and homogenized in 10 volumes (w/v) of 1.15% KCl. TBARS was measured according to the method described by Ohkawa et al. (1979). TBARS were calculated as nanomoles of thiobarbituric acid equivalent per milligram of protein.

Glutathione Assay. For determination of the glutathione levels, brain tissues from five rats were pooled and homogenized in 5% sulfosalicylic acid in an ice-water bath. Homogenates consisting of 100 mg tissue in 1 ml (10%) were centrifuged at 8000g for 10 minutes at 4°C to remove proteins. Supernatants were assayed for glutathione using GSSG/GSH Quantification Kit (Dojindo, Kumamoto, Japan) according to the manufacturer’s instructions.

Effects of PB and NAC Treatment on Ugt1a6, Ugt1a7, and Cyp2b1 Expression in Rat Hepatocytes. Hepatocytes were isolated from 5-week-old male Sprague-Dawley rats (n = 5) using a two-step collagenase perfusion method. Drug treatments were initiated 24 hours after plating. Hepatocytes were treated with 2 mM PB and/or 10 mM NAC for 72 hours and were collected for RNA preparation.

DNA Methylation Assay. Genomic DNA from pooled rat brains (n = 5) was extracted with phenol/chloroform followed by ethanol precipitation (Brilliant ...

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**Table 1** Primer sequences prepared for the Ugt standard solution

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Sequence</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ugt1a6</td>
<td>NM_057105</td>
<td>Sense 5'-GGGACACATGAGAATGACGAGG-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
<tr>
<td>Ugt1a7</td>
<td>NM_130407</td>
<td>Antisense 5'-CCCGGCACATTGAACTCA-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
<tr>
<td>CAR</td>
<td>NM_022941</td>
<td>Sense 5'-TGCTGCAACATGTCGCTGA-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
<tr>
<td>Nrf2</td>
<td>AF037350</td>
<td>Antisense 5'-GGGCGAGATGACGAGG-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
<tr>
<td>HO-1</td>
<td>NM_019240</td>
<td>Sense 5'-CTGGCCCTACATGACACTCA-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
<tr>
<td>β-actin</td>
<td>NM_031144</td>
<td>Antisense 5'-CCGCGACCATTGAACTCA-3'</td>
<td>100</td>
<td>Sakakibara et al. (2014)</td>
</tr>
</tbody>
</table>
et al., 1991) and was assayed for methylated DNA using Methylated DNA Quantification Kit (abcam) according to the manufacturer’s instructions.

**Histone Modification Assay.** Histone extracts from pooled rat brains (n = 5) were prepared using EpiSeeker Histone Extraction Kit (abcam). The histone extracts were assayed for histone modification using Histone H3 (tri-methyl K4), Histone H3 (tri-methyl K9), and Histone H3 (acetyl K9) Quantification Kits (abcam) according to the manufacturer’s instructions.

**Quantification of PB Concentrations.** The concentrations of PB in plasma and brain tissues were measured by high-performance liquid chromatography with tandem mass spectrometry. Brain tissue was homogenized in 10 volumes (w/v) of the homogenate buffer containing 10 mM Tris-HCl, 0.1 mM EDTA, and 20% glycerin. Fifty microliters of brain homogenate or plasma and 200 μl of the homogenate buffer containing 10 mM Tris-HCl, 0.1 mM EDTA, and 10% of g/ml barbital as internal standard were mixed. The acetonitrile mixtures were vortexed and then centrifuged at 9000 g for 10 minutes. Ten microliters of the supernatant obtained was subjected to high-performance liquid chromatography with tandem mass spectrometry. Liquid chromatography was performed using a Prominence instrument (Shimadzu Corporation, Kyoto, Japan) equipped with an Inertsil ODS-3 column (3.0 × 150 mm; GL Sciences, Shinjuku-ku, Tokyo, Japan). The column temperature was 40°C, and the flow rate was 0.2 ml/min. The mobile phase consisted of acetonitrile/10 mM ammonium acetate [40:60 (v/v)] and 50% acetonitrile containing 0.25 μg/ml barbital as internal standard were mixed. The acetonitrile mixtures were vortexed and then centrifuged at 9000 g for 10 minutes. Ten microliters of the supernatant obtained was subjected to high-performance liquid chromatography with tandem mass spectrometry. Liquid chromatography was performed using a Prominence instrument (Shimadzu Corporation, Kyoto, Japan) equipped with an Inertsil ODS-3 column (3.0 × 150 mm; GL Sciences, Shinjuku-ku, Tokyo, Japan). The column temperature was 40°C, and the flow rate was 0.2 ml/min. The mobile phase consisted of acetonitrile/10 mM ammonium acetate [40:60 (v/v)]. The liquid chromatographic column was connected to an API4000 tandem mass spectrometer operated in the negative electrospray ionization mode. The turbo gas was maintained at a temperature of 300°C. Mass/charge (m/z) ion transitions were recorded in the multiple-reaction monitoring mode, with m/z 231.0/187.8 for PB and m/z 182.7/139.8 for barbital. The limit of detection for PB was 1.7 pmol with a coefficient of variation of less than 10%. Data are shown as the mean values for five rats in each group.

**Statistical Analysis.** Statistical analyses were performed using Student’s paired t tests with the Kaleida-Graph computer program (Synergy Software, Reading, PA).

**Results**

**Effects of PB on Ugt1α6 and Ugt1α7 mRNA Expression in Rat Brains.** Constitutive expression of Ugt1α6 and Ugt1α7 mRNAs was observed in all nine regions, and the expression levels were 1.5–10% of the expression levels in the liver. Following treatment with PB, Ugt1α6 and Ugt1α7 mRNA levels were increased in the piriform cortex, hippocampus, medulla oblongata, olfactory bulb, striatum, and thalamus; the degree of induction differed among the nine regions examined in this study (Fig. 1). Ugt1α6 and Ugt1α7 mRNA levels were particularly increased in the striatum and thalamus (Ugt1α6, 3.0- and 2.9-fold, respectively; Ugt1α7, 2.6- and 2.6-fold, respectively). The degrees of induction of Ugt1α6 and Ugt1α7 were correlated (r = 0.92, p < 0.001).

**Effects of PB on APAP Glucuronidation in Rat Brains.** APAP glucuronidation, which is catalyzed mainly by Ugt1α6 and Ugt1α7, was determined in the medulla oblongata, striatum, and thalamus, where relatively high mRNA induction was observed. Following PB treatment, APAP glucuronidation in the medulla oblongata by 1.8-fold (control, 0.23 ± 0.01 pmol/min per milligram of protein; PB, 0.41 ± 0.01 pmol/min per milligram of protein). In the thalamus, APAP glucuronidation also increased by 1.2-fold (control, 0.19 ± 0.01 pmol/min per milligram of protein; PB, 0.23 ± 0.00 pmol/min per milligram of protein). However, APAP glucuronidation remained unchanged in the striatum (control, 0.23 ± 0.01 pmol/min per milligram of protein; PB, 0.22 ± 0.01 pmol/min per milligram of protein).

**Effects of PB on the Expression Levels of Nuclear Receptors in Rat Brains.** The constitutive expression levels of CAR and Nrf2 mRNAs were detected in all nine regions of the brain, with different degrees of expression (Fig. 2; Fig. 4A). Following treatment with PB, CAR mRNA was not changed in any of the brain regions, with the exception of the medulla oblongata (Fig. 2). Although the expression of the nuclear CAR protein was increased by 1.5-fold in the liver, it was not increased in the striatum or thalamus, where Ugt1α6 and Ugt1α7 mRNA levels were increased by PB (Fig. 3).

Following PB treatment, Nrf2 mRNA was increased in the hippocampus, medulla oblongata, olfactory bulb, striatum, and thalamus by 1.3-, 1.3-, 1.6-, 2.4-, and 2.0-fold, respectively (Fig. 4A). The degree of induction of Nrf2 mRNA by PB was correlated with that of Ugt1α6 and Ugt1α7 mRNAs (Ugt1α6, r = 0.97, p < 0.001; Ugt1α7, r = 0.90, p < 0.001) (Fig. 4B).

**Fig. 1.** Changes in Ugt1α6 (A) and Ugt1α7 (B) mRNA expression in response to PB treatment in rat brains. Each column represents the mean ± S.D. of three independent determinations. The mRNA expression levels were normalized to the expression of β-actin mRNA. **p < 0.01, ***p < 0.001 compared with the control.

**Fig. 2.** Expression levels of CAR mRNA in rat brains following PB treatment. The expression of CAR mRNA was calculated as the value relative to that of the control in the cerebellum. The mRNA expression level was normalized to the expression of β-actin mRNA. Each column represents the mean ± S.D. of three independent determinations. **p < 0.01 compared with the control.
Effects of PB on Oxidative Stress Markers in Rat Brains. The level of TBARS (lipid peroxidation) and total glutathione (nonenzymatic antioxidant defenses) were significantly increased in the medulla oblongata, olfactory bulb, striatum, and thalamus after PB treatment (Fig. 5, A and B). HO-1 mRNA (regulated by Nrf2) was increased in all nine regions (Fig. 6), and the fold changes of HO-1 mRNA correlated with those of Ugt1a6 and Ugt1a7 mRNA (Ugt1a6, $r = 0.84$, $p < 0.01$; Ugt1a7, $r = 0.89$, $p < 0.01$).

Effects of NAC on Ugt1a6, Ugt1a7, and Cyp2b1 mRNA Expression in Rat Hepatocytes Treated with PB. Following PB exposure for 72 hours, Ugt1a6 and Ugt1a7 mRNAs were increased by 1.9- and 1.7-fold, respectively, in rat hepatocytes (Fig. 7). NAC treatment decreased the induction of Ugt1a6 and Ugt1a7 mRNAs by 1.1- and 1.2-fold, respectively. PB treatment also increased HO-1 mRNA by 3.0-fold; this effect was inhibited by NAC. Cyp2b1, a representative target gene of CAR, was induced by PB and, unlike Ugt1a6 and Ugt1a7, the level of induction was not decreased by NAC treatment (Fig. 7).

Effects of PB on DNA Methylation and Histone Modifications in Rat Brains. Following treatment with PB, DNA methylation was unchanged in five brain regions (Fig. 8A). Histone H3 lysine 4 (H3K4) trimethylation was increased by 2.7- and 2.9-fold in the striatum and thalamus, respectively. (Fig. 8B).
thalamus, respectively (Fig. 8B), whereas histone H3 lysine 9 (H3K9) trimethylation was decreased by 0.34-fold in the thalamus (Fig. 8C). H3K9 acetylation was unchanged in the five regions (Fig. 8D).

Concentrations of PB in Rat Brains. The PB concentrations in the brain and plasma were quantified at 1 hour and 24 hours after the last treatment. There were no significant differences in PB concentrations in any brain region at 1 hour and 24 hours (Table 3).

Discussion

In the present study, we found that the expression levels of Ugt1a6 and Ugt1a7 mRNAs in the rat brain were increased by PB. The degree of induction differed among the nine examined brain regions, and those of Ugt1a6 and Ugt1a7 mRNAs were correlated. Therefore, the increases in Ugt1a6 and Ugt1a7 mRNA levels can be attributed to similar mechanisms.

PB is a typical inducer promoting the nuclear translocation of CAR, which is normally sequestered in the cytoplasm by the cytoplasmic CAR retention protein (Kobayashi et al., 2003). CAR translocates to the nucleus and heterodimerizes with the retinoid X receptor (RXR) α. The heterodimer then binds to a PB-responsive enhancer module in the region upstream of the promoter in CAR target genes, such as CYP2B, resulting in transcriptional activation (Honkakoski et al., 1998). In the present study, the expression of the nuclear CAR protein was increased in the liver following PB treatment, whereas the accumulation of CAR protein in the nucleus was not observed in the striatum and thalamus, where the expression levels of Ugt1a6 and Ugt1a7 mRNAs were increased (Fig. 3). In some brain regions, such as the cortex, hippocampus, striatum, and thalamus, PB and 2,4,6-tryphenyl dioxane did not affect CYP2B expression; in contrast, these agents caused a dramatic increase in CYP2B expression in the liver (Upadhya et al., 2002; Pustylnyak et al., 2009; Stamou et al., 2014). These results suggested that another mechanism, not involving CAR, may have contributed to the transcriptional activation of Ugt1a6 and Ugt1a7 in the rat brain.

Nrf2 is activated by oxidative/electrophilic stress and translocates to the nucleus from the cytosol, where it binds to the antioxidant response element with the small Maf protein and leads to transcriptional activation of target genes (Shen and Kong, 2009). Because there is an antioxidant response element in the region upstream of Nrf2 gene, Nrf2 is self-regulated in mice (Miao et al., 2005). Ugt1a6 and Ugt1a7 mRNAs are also increased through transcriptional activation by Nrf2 in mice and rats (Shelby and Klaassen, 2006; Buckley and Klaassen, 2009). In this study, we showed that Nrf2 mRNA was increased by PB and that the degree of induction was correlated with those of Ugt1a6 and Ugt1a7 mRNAs (Fig. 4B). Therefore, Ugt1a6 and Ugt1a7 mRNAs...
were regulated through the Nrf2 signaling pathway. In fact, the level of TBARS was increased in the brain regions where Ugt1a6 and Ugt1a7 mRNAs were increased following the PB treatment (Fig. 5A). These results suggested that oxidative stress was induced by PB in the rat brain, consistent with a previous study in rats (Reeta et al., 2011). The brain may be more susceptible to oxidative stress because it contains high levels of unsaturated fatty acids, which are the main targets of free radicals and cellular lipid peroxides. Measurement of TBARS is meaningful as an oxidative stress marker in the brain. Moreover, the level of total glutathione was also increased in some brain regions (Fig. 5B). Such increases in glutathione levels could result from the increase in γ-glutamylcysteine synthetase, which catalyzes the rate-limiting step in the de novo synthesis pathway of glutathione. The expression of γ-glutamylcysteine synthetase and the level of glutathione in rat neurons and glia cells are increased by oxidative stress through the Nrf2 signaling pathway (Iwata-Ichikawa et al., 1999; Shih et al., 2003).

Because the degree of induction of Ugt1a6 and Ugt1a7 mRNAs was correlated with the degree of induction of Nrf2-target gene HO-1, the oxidative stress induced by PB may activate Nrf2 signaling pathway. To verify this hypothesis, we examined the effects of the antioxidant NAC on the induction of Ugt1a6 and Ugt1a7 mRNAs by PB in rat hepatocytes. Our data showed that NAC exposure inhibited the induction of Ugt1a6, Ugt1a7, and HO-1 mRNAs but not Cyp2b1 mRNA (Fig. 7). These results indicated that Ugt1a6 and Ugt1a7 mRNAs were increased by oxidative stress derived from PB.

H3K4 trimethylation correlates with activation of gene expression, whereas H3K9 trimethylation is associated with gene silencing (Kimura, 2013). Moreover, H3K4 trimethylation is increased by oxidative stress (Niu et al., 2015). In the present study, H3K4 trimethylation was increased in the striatum and thalamus, and H3K9 trimethylation was decreased in the thalamus. Moreover, the degree of induction of Ugt1a6 and Ugt1a7 mRNAs by PB in the striatum and thalamus was higher than those in other regions. These results suggested that the regional differences in the degree of the induction in Ugt1a6 and Ugt1a7 may be associated with differences in epigenetic changes induced by PB in the rat brain.

From the above results, we hypothesized that oxidative stress generated by PB and histone modifications may be involved in the transcriptional activation of Ugt1a6 and Ugt1a7; however, the degrees of induction were not associated with the concentrations of PB in each of the nine brain regions (Table 3). Capacities and responses to oxidative stress among the nine brain regions may be varied, which would be expected to cause differences in the transcriptional activation of Ugt1a6 and Ugt1a7 among the nine brain regions.

In the present study, PB increased not only Ugt1a6 and Ugt1a7 mRNAs but also APAP glucuronidation in brain regions with relatively high induction of mRNA. Ugt1a6 and Ugt1a7 are mainly involved in the conjugation of planar phenols, including endogenous compounds (e.g., serotonin) and drugs such as APAP, as well as metabolites of carcinogens, such as benzo(a)pyrene-3,6-diphenol (Bock and Köhle 2005). Therefore, Ugt1a6 and Ugt1a7 in the brain may play important roles in detoxification and homeostasis to protect the brain. In the present study, APAP glucuronidation was increased by PB in the

| TABLE 3 |

Concentrations of PB in the brain and plasma at 1 hour and 24 hours after administration

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>PB(μg/mg protein)</th>
<th>1 hour (μg/ml)</th>
<th>24 hours (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>291.1 ± 32.0</td>
<td>9.0 ± 6.3</td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>320.1 ± 34.0</td>
<td>9.1 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>307.2 ± 23.7</td>
<td>9.1 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>324.2 ± 34.5</td>
<td>8.9 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>311.8 ± 19.4</td>
<td>9.6 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>322.5 ± 27.5</td>
<td>9.7 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>306.7 ± 30.9</td>
<td>9.1 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td>320.3 ± 35.6</td>
<td>8.9 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>325.7 ± 29.9</td>
<td>9.8 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>76.1 ± 9.5^</td>
<td>2.8 ± 1.7^</td>
<td></td>
</tr>
</tbody>
</table>

^μg/ml.
medulla oblongata and thalamus, where serotonin, a Ugt1a6 sub-
strate, was found (Icemam et al., 2013; Chung et al., 2015). Thus, the
concentrations of UGT substrates, including serotonin, may change
locally, thereby altering efficacy. However, the increase in APAP
glucuronidation was not correlated with the increase in Ugt1a6 and
Ugt1a7 mRNA expression levels in the striatum. A previous report
showed that human UGT1A mRNA and protein levels did not always
correlate with each other (Izukawa et al., 2009; Oda et al., 2012;
Ohtsuki et al., 2012), suggesting that the level of induction of Ugt
mRNA does not correlate with the induction of glucuronidation
activities. To determine changes in Ugt1a6 and Ugt1a7 enzyme
activities in detail may require more specific Ugt1a6 and Ugt1a7
substrates. Similar to previous reports, our data suggested that an
unexplained epigenetic mechanism may be involved in the post-
transcriptional regulation of Ugt1a6 and Ugt1a7 in the rat brain.
UGTs play important roles in the detoxification of xenobiotics,
including drugs and environmental substances, protecting the organ-
ism from the effects of hazardous compounds. UGTs also function as
antioxidants in their role of maintaining the redox balance (Kalthoff
et al., 2010). Compared with other organs, the brain appears especially
susceptible to excessive oxidative stress (Dringen et al., 2000; Bergamini
et al., 2004). Hence, the transcriptional activation of UGT isoforms
in the brain contributes to the protection of the brain against
cytotoxicity.
In summary, we found that PB induced the expression of Ugt1a6 and
Ugt1a7 in rat brains. These changes could affect pharmacological and
physiologic functions owing to alterations in the concentrations of
the drugs in the brain.

Authorship Contributions
Participated in research design: Sakakibara, Katoh, Nadai. Conducted experiments: Sakakibara, Katoh, Kondo. Wrote or contributed to the writing of the manuscript: Sakakibara, Katoh, Nadai.

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Sakakibara et al.

Sakakibara et al.


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