unknown. Based on our findings in mice, we propose that the impairment is attributable to the untimely enhancement of embryonic neurogenesis, which leads to depletion of the NPC pool and consequently to a decreased level of postnatal neurogenesis in the hippocampus. Children of epileptic expectant mothers treated with VPA may also have hippocampal neurons with abnormal morphology and activity, as were observed in this study. Although prenatal exposure to AEDs such as VPA may have detrimental effects that persist until adulthood, we suggest that these effects could be mitigated by a simple physical activity such as running. Our results thus offer a straightforward strategy to help children born to epileptic mothers.

The cognitive deficits associated with prenatal VPA exposure might not due solely to the reduced neurogenesis with the abnormal neuronal morphology in the hippocampus, and there is a possibility that the low freezing responses in fear associative tests were contributed by the deficiency in amygdala, nociception, and/or motoric functions. Nevertheless, our data suggested that the reduced neurogenesis associated with the abnormal neuronal morphology in the hippocampus were very likely to be correlated with the observed cognitive deficits for several reasons. First, voluntary running is well known for its effect on enhancing both adult neurogenesis in the DG of the hippocampus and hippocampus-dependent learning and memory (Zhao et al., 2008), and this voluntary running could recover the cognitive deficit, if not all, in VPA-treated mice with reduced neurogenesis in the DG. Second, to the best of our knowledge, there are no reports to date that show a direct contribution of voluntary running to the enhancement of amygdala function that subsequently leads to an improvement in the cued fear response. Third, based on experiments that we have conducted, we could not find any significant differences in amygdala size and in the expression levels of cortical layer-specific genes of MC- and VPA-treated mice, with or without voluntary running. Fourth, total traveled distance in the open field, elevated plus and the Y-maze tests, and the number of light/dark transitions were not significantly different between MC- and VPA-treated mice, although in our earlier experiment some of these parameters showed modest differences. Moreover, in fear associative test, VPA-treated mice move similarly to MC-treated mice before the start of the tone (pre-tone), which indicate that motor deficiency is unlikely to be the main cause of low freezing responses in VPA-treated mice. Fifth, MC- and VPA-treated mice have similar basal nociceptive response and startle response to electric footshock during the conditioning for fear-associative test, thus it seems unlikely that VPA-treated mice have abnormal nociception and cannot sense the foot shock. Taking these facts into consideration, we therefore suggested that the reduced neurogenesis associated with the abnormal neuronal morphology in the hippocampus were very likely to be a critical cause of the observed cognitive deficits. However, we still cannot completely exclude the possibility that changes in other brain areas may also contribute to the deficits, warranting further future investigation.

We and others have shown previously that VPA treatment induces neuronal differentiation but suppresses glial differentiation of cultured multipotent NPCs (Hsieh et al., 2004; Balasubramaniyan et al., 2006; Murabe et al., 2007; Abematsu et al., 2010; Juliandi et al., 2012). We have now demonstrated that VPA also increases histone acetylation in the embryonic forebrain and induces neuronal differentiation of embryonic NPCs. Previous study have shown that VPA promotes neuronal differentiation by increasing histone H4 acetylation at proneural gene promoters (Yu et al., 2009). However, several studies have suggested that the activation of GSK-3β/β-catenin and/or ERK pathway is the main cause for the increase neurogenesis of NPCs by VPA (Yuan et al., 2001; Jung et al., 2008; Hao et al., 2004; Go et al., 2012). It has been suggested that VPA might have various cellular effects that will depend on the context of VPA usage and/or cell type and experimental design used in the study, which warrant further research to reveal the connection between these effects (Kostrouchová et al., 2007; Rosenberg, 2007).

We suggest that gene expression change caused by VPA is attributable mainly to its HDAC-inhibiting activity. To date, more than a dozen HDACs have been characterized and they are classified into at least three major groups. In particular, HDAC1 and HDAC2, belonging to the class I group, have been reported to regulate NPC differentiation (Sun et al., 2011). NPCs express high levels of HDAC1 and some of them also express low levels of HDAC2 (MacDonald and Roskams, 2008). Interestingly, as NPCs are committed to the neuronal lineage, expression of HDAC2 is upregulated while that of HDAC1 is downregulated and becomes undetectable in most post-mitotic neurons (MacDonald et al., 2005; MacDonald and Roskams, 2008); on the other hand, HDAC1 expression is sustained in the majority of cells in glial lineages (astrocytes and oligodendrocytes), in which HDAC2 is not detected (Shen et al., 2005; MacDonald and Roskams, 2008). Moreover, HDAC2, but not HDAC1, was found to inhibit astrocytic differentiation (Humphrey et al., 2008). Therefore, although VPA is capable of inhibiting both HDAC1 and HDAC2 (Kazantsev and Thompson, 2008), it is tempting to speculate that the main target of VPA in HDAC inhibition-mediated neuronal differentiation of NPCs is HDAC1. It will be of interest to explore this possibility in a future study.

Neurogenesis in the adult mammalian brain occurs throughout life and has been clearly demonstrated at two

locations under physiological conditions: the SVZ of the lateral ventricle and the subgranular zone (SGZ) of the DG in the hippocampus (Alvarez-Buylla and Lim, 2004). Several studies have shown that hippocampal neurogenesis is regulated by both physiological and pathological activities at different stages, including (1) proliferation of NPCs, (2) fate determination and differentiation of NPCs, and (3) survival, maturation, and integration of newborn neurons (Zhao et al., 2008). Furthermore, each of these stages is subject to regulation by numerous intrinsic and extrinsic factors (Suh et al., 2009). Genetic and environmental factors that affect adult hippocampal neurogenesis also cause alteration in cognitive performance, suggesting roles for adult hippocampal neurogenesis in learning and memory (Zhao et al., 2008). Our results showed that VPA-treated mice have a decreased level of postnatal neurogenesis in the hippocampus, which correlates with their poor performance in learning and memory tests. We have shown here and elsewhere (Hsieh et al., 2004; Jessberger et al., 2007) that VPA can reduce the proliferation of NPCs, and this reduction, together with the enhancement of neurogenesis, probably led to the depletion of the NPC pool in VPA-treated mice. It is possible that this depletion caused a slower differentiation of the residual NPCs in order to maintain required number of NPC pool during life. This possibility is an interesting avenue to be explored in the future.

In accordance with previous studies (van Praag et al., 1999a, 1999b), we found that voluntary running augments hippocampal neurogenesis of both MC- and VPA-treated mice, and it restores learning and memory deficiencies in VPA-treated mice. A previous report has shown the same restoration of decreased hippocampal neurogenesis and learning deficits in aged rodents by voluntary running (van Praag et al., 2005), although the precise molecular mechanisms responsible for voluntary running-induced neurogenesis remain undetermined (Deng et al., 2010). Here, we propose that at least the increase expression level of *Bdnf*, and the reduction of activated microglia may contribute to the restoration of impaired hippocampal neurogenesis and neuronal morphology in the DG of VPA-treated mice after voluntary running. However, future exploration is necessary to reveal the direct connection between the increase expression level of *Bdnf* and the reduction of microglia and its activated form in the hippocampus after voluntary running.

EXPERIMENTAL PROCEDURES

Animal Treatment

All experiments were carried out according to institutional animal experimentation guidelines, which comply with the NIH Guide for Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. Pregnant C57BL/6 mice were individually housed in plastic breeding cages with free access to water and pellet diet in a 12-hr light-dark cycle. For a detailed description of groups and treatments, see the Supplemental Experimental Procedures.

Immunohistochemistry, Nissl, and Golgi Staining

Mice were anesthetized and perfused with PBS followed by 4% PFA in PBS. The brain was dissected, postfixed, and processed for immunohistochemistry. For Nissl staining, brain sections were defatted with xylene, hydrated through a graded ethanol series (100%, 95%, and 70%), and washed with water before stained with 0.2% thionin solution (pH 4.0). Sections were then dehydrated in water and a graded ethanol series (70%, 95%, and 100%), clear in xylene, and mounted with Entellan (Merck). For Golgi staining, the brain was removed from the skull without any perfusion and then sectioned (100 μm) on a cryostat. For a more detailed description and list of antibodies, see the Supplemental Experimental Procedures.

Measurement and Morphometrics

For a detailed description of cell count, volume measurement and cell/tissue morphometrics, see the Supplemental Experimental Procedures.

Gene Expression Analysis

For a detailed description of GeneChip and real-time qPCR procedures, see the Supplemental Experimental Procedures.

Behavioral Tests

Behavioral experiments were performed sequentially using male mice. For a more detailed description, see the Supplemental Experimental Procedures.

Voltage-Sensitive Dye Imaging

Experiment was done using hippocampal slices. For a more detailed description, see the Supplemental Experimental Procedures.

Statistics

Statistical analyses were performed by Student's two-tailed t test (unpaired) and one-way or two-way ANOVA using R software (http://www.r-project.org) (n indicates individual mice).

ACCESSION NUMBERS

The accession number for the GeneChip data reported in this paper is GEO: GSE42904.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2015.10.012.

AUTHOR CONTRIBUTIONS

B.J. and K.N. conceived and designed the study. B.J., K. Tanemura, K.I., T.T., Y.F., M.O.I., N.M., and D.I. carried out the experiments. B.J., K. Tanemura, K.I., T.T., M.A., T.S., K. Tsujimura, M.N., and K.N. analyzed the data. J.K. supported the experiments. B.J. and K.N. wrote the manuscript.

ACKNOWLEDGMENTS

We thank Y. Bessho, T. Matsui, Y. Nakahata, J. Kohyama, T. Takizawa, M. Namihira, S. Katada, and T. Imamura for valuable discussions. We also thank I. Smith for critical reading of the manuscript. We are very grateful to M. Tano for her excellent secretarial assistance and other laboratory members for discussion and technical help. This research was supported in part by the NAIST Global COE Program (Frontier Biosciences: Strategies for survival and adaptation in a changing global environment) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); a Grant-in-Aid for Scientific Research on Innovative Area: Neural Diversity and Neocortical Organization from MEXT; Health Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan; Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Corporation; and Research Fellowships for Young Scientists from the Japan Society for the Promotion of Science.

Received: June 24, 2015
Revised: October 22, 2015
Accepted: October 23, 2015
Published: November 19, 2015

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FULL PAPER *Toxicology*

Weak activity of UDP-glucuronosyltransferase toward Bisphenol analogs in mouse perinatal development

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(Received 2 April 2015/Accepted 4 June 2015/Published online in J-STAGE 15 June 2015)

ABSTRACT. Bisphenol A (BPA) is a widely used industrial chemical that disrupts endocrine function. BPA is an endocrine disrupting chemical (EDC) that has been demonstrated to affect reproductive organ development, brain development, metabolic disease and post-natal behavior. Accordingly, Bisphenol analogs, Bisphenol F (BPF, bis (4-hydroxyphenyl) methane) and Bisphenol AF (BPAF, 4,4-hexafluoroisopropylidene) diphenol) are used as replacements for BPA. BPA is mainly metabolized by UDP-glucuronosyltransferase (UGT), UGT2B1, but this effective metabolizing system is weak in the fetus. In the present study, we demonstrated that hepatic UGT activity toward BPAF was very weak, in comparison with BPA and BPF, in the fetus, pups and dams. Conversely, hepatic UGT activity toward BPF was very weak in the fetus and newborn pups, and was increased to the same level as BPA post-partum. In conclusion, BPAF possibly tends to accumulate in the fetus, because of weak metabolism during the perinatal period, suggesting that the metabolism of individual Bisphenol analogs requires assessment to properly gauge their risks.
KEY WORDS: Bisphenol A, Bisphenol AF, Bisphenol F, UDP glucuronosyl transferase (UGT)

doi: 10.1292/jvms.15-0197; *J. Vet. Med. Sci.* 77(11): 1479–1484, 2015

Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane) is an industrial chemical widely used in the manufacture of polycarbonate plastics and epoxy resin liners for aluminum cans [27, 33]. BPA is an endocrine disrupting chemical (EDC) that has been demonstrated to affect reproductive organ development, brain development, metabolic disease and post-natal behavior [4]. These adverse effects are thought to be due to disturbed signaling mechanisms involving estrogen, androgen and thyroid hormone. BPA is metabolized by phase II enzymes in rat liver, such as glucuronidation mediated by UDP-glucuronosyltransferase (UGT, Enzyme Classification 2.4.1.17), mainly UGT2B1 in rat liver [28]. Glucuronidation is the main pathway by which BPA is metabolized to a hydrophilic form lacking estrogenic activity. We were the first to report that BPA is highly glucuronidated in the rat liver [36]. We also reported that this metabolic system is weak in the fetus, due to low UGT2B1 expression in fetal rat liver [24, 26]. In recent years, numerous studies in rodents have reported that maternal BPA exposure causes adverse effects in the offspring [4, 34]. Consequently, regulatory agencies in some countries have begun to restrict the use of BPA. In 2010, the Canadian Government prohibited the importation, sale and advertising of polycarbonate baby bottles containing BPA (Government of Canada, 2010), and in 2011, the European Union prohibited the use of BPA in the manufacture of polycarbonate feeding bottles for infants (The European Commission, 2011).

Bisphenols are a class of chemicals with 2 hydroxyphenyl functionalities, which contain 2 benzene rings separated by 1 central carbon atom and include several analogs, such as Bisphenol B (BPB, 2,2-bis (4-hydroxyphenyl) butane), Bisphenol F (BPF, bis (4-hydroxyphenyl) methane), Bisphenol AF (BPAF, 4,4-hexafluoroisopropylidene) diphenol) and Bisphenol S (BPS, bis (4-hydroxyphenyl) sulfone) [29]. Because of worries associated with the adverse effects of BPA, the use of other bisphenol analogs as a replacement has increased [19, 20]. These structural analogs of BPA have been detected in beverages, canned foodstuffs and human serum [3, 19]. BPF and BPAF have weak estrogenic activity and have been found in dental materials [9], canned foods [19] and indoor dust [20]. BPAF has been shown to induce estrogenic effects through binding to the estrogen receptor (ER) [17, 18]. BPAF exhibits agonist activity toward ERα and antagonist activity toward ERβ [25]. BPAF was also reported to be an agonist of the human pregnane X receptor [31]. BPF shows an affinity for estrogen receptors and anti-androgenic activity *in vitro*, which is slightly different from BPA [15, 27, 29]. It was reported that BPF and BPAF are metabolized by UGT in human and rat liver [6, 16], and that BPF could cross the placental barrier at the late stage of gestation in rats [1]. Thus, bisphenol analogs behave and are metabolized in a manner similar to BPA. Therefore, there is a concern that BPA causes adverse effects in offspring,

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however, little is known about the risks associated with these structural substitutes, especially their influence on offspring. We propose that one of the mechanisms responsible for the adverse effects caused by bisphenols is weak UGT activity in the fetus and pups during the perinatal period.

In the present study, we evaluated risk during the perinatal period by examining UGT activity for BPA, BPF and BPAF in fetal, pup and maternal mouse liver and compared activity to UGT2B1 gene expression.

MATERIALS AND METHODS

Chemicals: Bisphenol A and high-performance liquid chromatography (HPLC) grade methanol were purchased from Kanto Chemical Co. (Tokyo, Japan). The glucuronide metabolites of bisphenol analogs were purified from bile after rat liver perfusion with 7.5 μmole Bisphenol A, Bisphenol F and Bisphenol AF [11] and were quantified by HPLC by determining the difference between untreated and beta-glucuronidase-treated samples, which were used as standards. All other reagents were of the highest grade available.

Animals: Ten-week old C57BL/6 mice (pregnant and neonatal mice with mothers) were purchased from Sankyo Lab Co. (Tokyo, Japan). Animals were housed individually under a 12/12-hr light/dark cycle and had *ad libitum* access to water and food. All of the animals were treated according to the Laboratory Animal Control Guidelines at Rakuno Gakuen University, which are based on the Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes (ethics committee protocol approval number ES23A07, approved Jan 13, 2012).

Tissue collections: Dams and neonatal mice were euthanized by incision of the caudal vena cava under anesthesia (intraperitoneal injection of pentobarbital). After euthanasia, each liver was dissected and divided into 2 pieces for analysis of enzyme activity and gene expression. Four liver samples were collected from male and female offspring from individual dams. Four liver samples from mothers were also isolated at each stage. The livers for gene expression analysis were treated with RNAlater (Qiagen, Hilden, Germany) and stored at −80°C. The livers used for enzyme analysis were homogenized in 4 vol. of 0.15 M KCl solution containing 1 mM EDTA. The homogenates were centrifuged for 30 min at 9,000 g, and the supernatant fractions were centrifuged at 105,000 g for 60 min to obtain microsomes. Purified microsomes were stored at −80°C until analysis. The dissolved microsomes were activated by incubation with 0.01% sodium cholate for 30 min at 0°C.

Analysis of UGT activity: UDP-glucuronosyltransferase activity toward BPA substrates was assayed in a 400 μl volume containing 0.1 M Tris-HCl buffer (pH7.4), 5 mM $MgCl_2$, 0.2 mM BPA, 3 mM UDP glucuronic acid and 10 mg microsomal protein. Protein concentration was determined by the method of Lowry *et al.* [21]. The mixture was incubated at 37°C for 30 min and then boiled for 5 min to stop the reaction. After boiling, the mixture was centrifuged at 12,000 g, and the supernatant was filtered using a disposable disc filter (HLC-DISK3, Kanto Chemical Co.). Each supernatant was then analyzed using a dual pump (DP-8020) HPLC system (Tosoh Corp., Tokyo, Japan) with a fluorescent photometer (FS-8020) and a column oven (CO-8020). Samples were separated at 40°C using a Unison UK-C18 reverse-phase column (2.0 mm i.d. × 150 mm; Imtakt Corp., Kyoto, Japan) at a flow rate of 1.0 m*l*/min under a linear gradient of solution A (methanol/water=24/76 vol/vol with 10 mM ammonium acetate, pH=7.0) and solution B (methanol) for 20 min. Glucuronides were detected at excitation/emission of 275/308 nm. The results were recorded using LC-8020 integration software (Tosoh Corp.); the elution peaks of BPA and its conjugates were noted, and the concentrations were compared with the standards. The detection limit of assay was 0.2 μM.

To identify the metabolites, LC-ESI-TOF/MS analysis was performed using a LCT premier XE (Waters Corp.). The eluted peaks in the HPLC analysis described above were collected and infused at a flow rate of 0.2 m*l*/min into the LC-ESI-TOF/MS using IC-3100 syringe driver (Matsuura Corp., Tokyo, Japan). BPA glucuronides were monitored in negative-mode (data not shown).

Analysis of gene expression: Total RNA for RT-PCR was isolated using an RNeasy mini kit and RNase-free DNase 1 (Qiagen) from tissues treated with RNAlater (Qiagen), according to the manufacturer's instructions. Total RNA was converted to cDNA using a ReverTra Ace (TOYOBO) reverse transcriptase and Oligo dT primers (TOYOBO), according to the manufacturer's instructions. Quantitative analysis of *UGT2B1* mRNA was normalized to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). PCR was performed with the following primers accordingly to a previous report [12]: *UGT2B1* sense primer, 5′- AGATGATGGGGAAGGCAGAT-3′, and *UGT2B1* antisense primer, 5′- GCAAGAGCAGAAGCAACTAC-3′; *UGT1A6* sense primer, 5′-CCTCAGTGAACGCGGACACGAC-3′, *and UGT1A6* antisense primer, 5′- TTCCTGTACTCTCTTAGAGGAGCCA-3′, *GAPDH* sense primer, 5′-TTCAACGGCACAGTCAAG-3′, and *GAPDH* antisense primer, 5′-CACACCCATCACAAACAT-3′. The nucleotide sequences for these primers were designed using DNA sequences obtained from GenBank. PCR amplification was performed as follows: denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec and extension at 72°C for 1 min for 35 cycles. *UGT2B1* and *UGT1A6* were amplified from diluted cDNA and used for quantitative RT-PCR analysis. The amplified fragments for each gene were cloned into the pSTBlue-1 Acceptor Vector (MERCK MILLIPORE) and subsequently transformed into *Escherichia coli* DH5α. Plasmid vectors with each of the appropriate genes were prepared and quantified using a spectrophotometer. A standard curve for each gene was produced using 100 or 10-fold serial dilutions of the template (10^8–10^2 copies). The reaction was performed using a Quantitect SYBR Green PCR kit (Qiagen) and iQ5/MyiQ Single-Color (Bio-Rad Laboratories), following the manufacturer's instructions. The correlation coefficient for UGT1A6, UGT2B1 and GAPDH were 1.000, 0.981 and 0.993, respectively. The amplification efficiency for UGT1A6, UGT2B1 and GAPDH

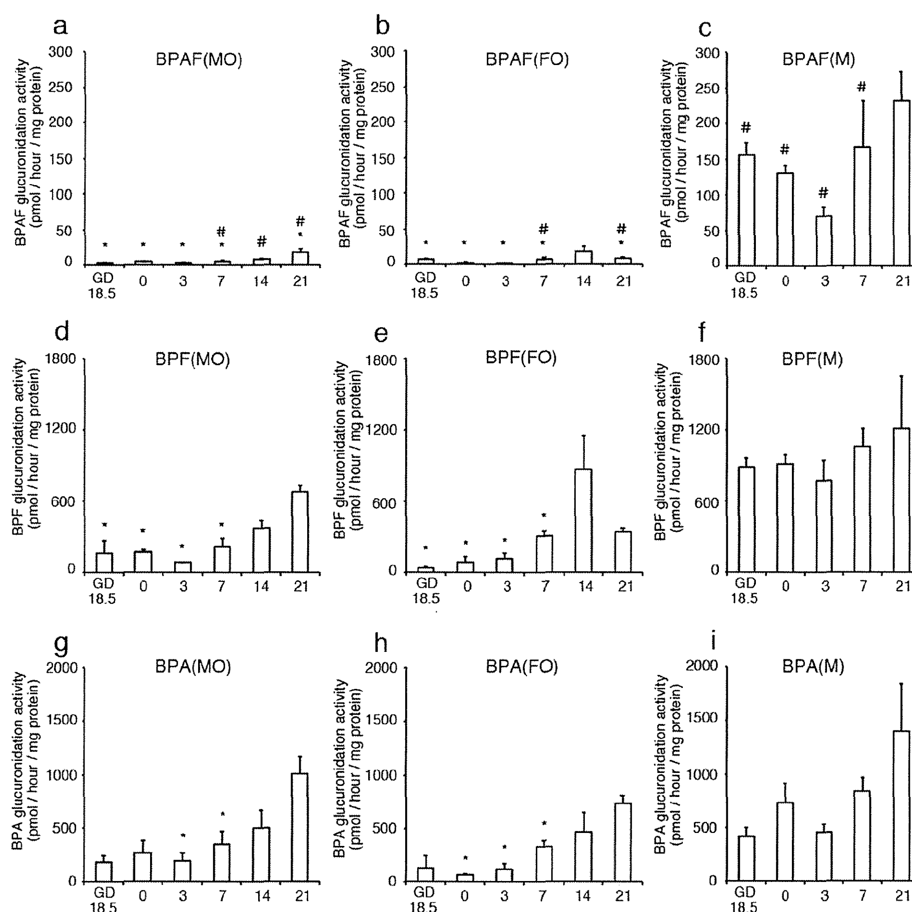

Fig. 1. UGT activity toward BPAF, BPF and BPA in mouse liver during perinatal development. UGT activity in male offspring (MO: a, d and g), female offspring (FO: b, e and h) and mothers (M: c, f and i) toward BPAF (a, b and c), BPF (d, e and f) and BPA (g, h and i) are shown from gestation day (GD) 18.5 to post-natal day 21. UGT activity values are shown as means ± S.E. In each chemical, the significant differences of UGT activity of male and female for mother in each stage were shown in an asterisk ($P<0.05$). The significant differences of UGT activity toward BPAF compared with both BPA and BPF in each stage were shown in # ($P<0.05$).

were 87.3%, 80.1% and 115%, respectively. The copy number of each gene expressed in the liver was calculated from their respective standard curves and normalized to that of *GAPDH* (Fig. 2). Quantitative values are presented as mean ± SE (n=4).

Statistical analysis: All data were presented as the mean ± S.E., and the means were compared by use of ANOVA, with the P value of 0.05 as the level of significance.

RESULTS

In this study, UGT activity toward BPA, BPF and BPAF was examined in hepatic microsomes purified from perinatal mice and mothers (Fig. 1). There were no sex differences in the enzymatic activity for each chemical. Intriguingly, UGT activity toward BPAF was extremely low compared with BPA and BPF in fetuses, pups and dams (Fig. 1a–1c). Furthermore, the activity was significantly low compared with mother at developmental stages (GD18.5-PD7 in Fig. 1a–1c), and activity did not increase to levels similar to that of the dams in newborn mice (Fig. 1a–1c PD7-21).

In comparison, hepatic UGT activity toward BPF was significantly weak in fetuses and newborn pups, and increased to levels similar to those for BPA after birth (Fig. 1d–1i). Furthermore, similar levels of UGT activity toward BPF and BPA were observed during pup development and in dams.

Hepatic UGT2B1 expression was statistically significantly low during the perinatal period (less than 5.5% of dams) and increased from PD3 (about half level of dams), becoming similar to levels observed in the dams by PD 7 (Fig. 2A). On the other hand, hepatic UGT1A6 expression was slightly low (about half level of dams) during the perinatal period (Fig. 2B). Accordingly, it was thought that BPF was mainly metabolized by UGT2B1, like BPA.

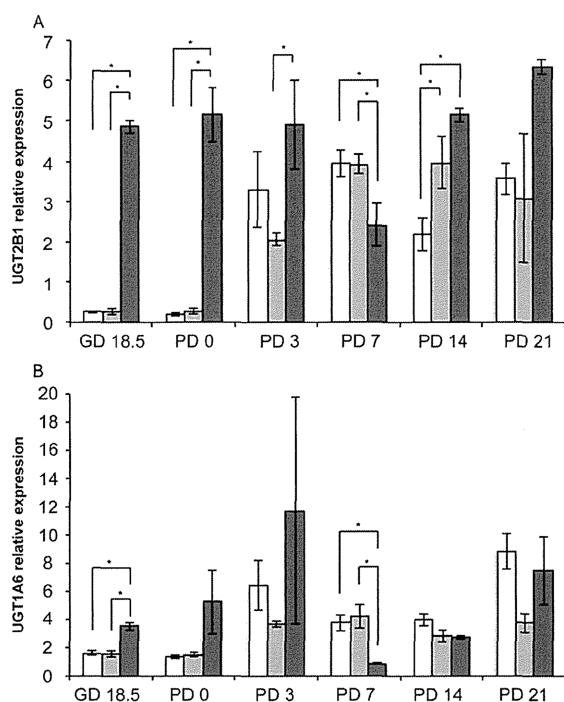

Fig. 2. Quantitative analysis of UGT2B1 and UGT1A6 gene expression by real-time RT-PCR. Quantitative analysis data of UGT2B1 (A) and UGT1A6 (B) mRNA expression in the liver of male offspring (white), female offspring (light gray) and mother (dark gray) are shown from gestation day (GD) 18.5 to post-natal day 21. The mRNA levels were normalized to GAPDH. Relative expression values are shown as means ± S.E. The significant differences of expression in each stage were shown in an asterisk ($P<0.05$).

DISCUSSION

UGT facilitates the excretion of endogenous and xenobiotic compounds, such as bilirubin, steroids and environmental pollutants, as water-soluble conjugates [22]. UGT1A1 mainly catalyzes the glucuronidation of bilirubin, and the absence of UGT1A1 causes Crigler-Najjar syndrome [13]. UGT1A6 and UGT1A7 catalyze the glucuronidation of similar substrates, such as planar phenols and benzo[a]pyrene [22]. UGT2B1 catalyzes the glucuronidation of a number of xenobiotics and endobiotics (steroids), such as estrogen and morphine [22].

Previously, we reported that BPA is highly glucuronidated primarily by UGT2B1 in the rat liver [10, 11, 36]. We also demonstrated that BPA-GA is transferred into the fetus by uterine perfusion, and we detected not only BPA-GA but also deconjugated BPA in the fetus and amniotic fluid [26]. Therefore, elucidation of the pharmacokinetics of bisphenols during the perinatal period is essential to recognizing the mechanism of adverse effects in offspring.

It was reported that BPAF is mainly metabolized to a glucuronide-conjugated metabolite in SD rats and that human metabolism is primarily mediated by UGT2B7 [16]. UGT2B7 is known to be an ortholog of the rodent UGT2B1 gene [22] and is weakly expressed in human fetal liver [14]. UGT2B1 was also weakly expressed in rodent fetuses and newborn pups [23, 26]. There are some reports of BPAF toxicity in recent years. BPAF has been shown to induce estrogenic effects *in vitro* [17, 18], exhibiting agonist activity toward ERα and antagonist activity toward ERβ [25]. BPAF has also been reported to be an agonist of the human pregnane X receptor [31]. Exposure to BPAF disrupts hormonal balance and gene expression in the hypothalamus-pituitary-gonad axis and liver, and also decreases egg fertilization in offspring of zebrafish [30, 35] and rats [8], possibly due to sperm deterioration.

As a result, it is thought that BPAF has high toxicity, despite being considered a safe replacement for BPA. This may be attributable, at least in part, to the extremely low perinatal BPAF metabolism observed here.

In comparison, hepatic UGT activity toward BPF was very weak in fetuses and newborn pups, and increased to levels similar to those for BPA after birth (Fig. 1D–1I). Accordingly, it was thought that BPF was mainly metabolized by UGT2B1, like BPA. Further works are needed to clarify kinetic properties by using isolated UGT isoenzyme mainly metabolizing BPAF. Some reports have shown that BPF is easily metabolized to BPF-glucuronide and BPF-sulfate in HepG2 and human primary hepatocytes [2, 6]. It was also shown that BPF is efficiently absorbed through the oral route and distributed to the reproductive tract in pregnant rats, and its residue passed the placental barrier at the late stage of gestation [1]. Both animal [5, 32] and human [7, 37] studies have demonstrated that BPA and BPA-GA are detected in fetuses and amniotic fluid, suggesting the presence of a placental transfer mechanism. We also demonstrated that BPA-GA is transferred into the fetus following uterine perfusion and that BPA-GA and deconjugated BPA are detected in the fetus and amniotic fluid [26]. These reports suggest that maternal and perinatal metabolism, as well as the pharmacokinetics of placenta transfer to the fetus, should be taken into consideration when assessing adverse effects in offspring caused by bisphenol analogs. UGTs are well known to be induced by some chemicals. Nobody knows UGT induction by BPAs. In future, we will have to confirm whether BPAs induce the UGT activity or not.

In summary, we demonstrated that hepatic UGT activity toward BPAF was very low compared with BPA and BPF in fetuses, pups and dams. UGT activity toward BPF was also found to be weak in perinatal offspring, in a manner similar to BPA. Further studies are required to clarify the pharmacokinetics of bisphenol analog transfer from mother to fetuses and pups to elucidate the mechanisms of adverse effects on subsequent generations.

AKNOWLEDGMENTS. This study was supported in part by the Health Science Research Grant H23-Kagaku-004 from the Ministry of Health, Labour and Welfare, Japan, and a Feasibility Study among Fundamental Studies under the framework of EXTEND 2010 (Extended Tasks on Endocrine

Disruption 2010) funded by the Ministry of the Environment, Japan.

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