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Development of Prostaglandin Endoperoxide Synthase Expression in the Ovine Fetal Central Nervous System and Pituitary

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Abstract

In this study, we tested the hypothesis that prostaglandin endoperoxide synthase -1 and -2 (PGHS-1 and PGHS-2) are expressed throughout the latter half of gestation in ovine fetal brain and pituitary. Hypothalamus, pituitary, hippocampus, brainstem, cortex and cerebellum were collected from fetal sheep at 80, 100, 120, 130, 145 days of gestational age (DGA), 1 and 7 days postpartum lambs, and from adult ewes (n=4–5 per group). mRNA and protein were isolated from each region, and expression of Prostaglandin Synthase -1 (PGHS-1) and -2 (PGHS-2) were evaluated using real-time RT-PCR and western blot. PGHS-1 and -2 were detected in every brain region at every age tested. Both enzymes were measured in highest abundance in hippocampus and cerebral cortex, and lowest in brainstem and pituitary. PGHS-1 and -2 mRNA's were upregulated in hypothalamus and pituitary after 100 DGA. The hippocampus exhibited decreases in PGHS-1 and increases in PGHS-2 mRNA after 80 DGA. Brainstem PGHS-1 and -2 and cortex PGHS-2 exhibited robust increases in mRNA postpartum, while cerebellar PGHS-1 and -2 mRNA's were upregulated at 120 DGA. Tissue concentrations of PGE₂ correlated with PGHS-2 mRNA, but not to other variables. We conclude that the regulation of expression of these enzymes is region-specific, suggesting that the activity of these enzymes is likely to be critical for brain development in the late-gestation ovine fetus.

Keywords

parturition; prostanoid; neuroendocrinology; fetus; HPA axis; brain

1. RESULTS AND DISCUSSION

Prostaglandins are involved in many physiological processes such as inflammation, response to pain, and vascular regulation (Morita, 2002; Smith et al., 2000). Generated within the fetal central nervous system, prostanoids modulate both basal- and stress-induced secretion of adrenocorticotropin (Reimsnider and Wood, 2005; Reimsnider and Wood, 2006; Tong et al., 1998), modulate blood pressure regulatory systems (Reimsnider and Wood, 2006) and influence fetal breathing movements (Adamson et al., 1997). Both PGHS-1 and -2 are expressed in the fetal brain (Tong et al., 2002; Deauseault et al., 2000; Norton et al., 1996), and there is evidence that prostanoids are synthesized within the fetal central nervous system

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(Pace-Asciak and Rangaraj, 1976). PGHS-1 and/or PGHS-2 expression in the CNS of the fetal sheep are modified by cerebral hypoperfusion (Tong et al., 1999), chronic blockade of fetal CNS estrogen receptors (Schaub et al., 2008), and chronic blockade of PGHS-2 activity with nimesulide (a specific inhibitor of PGHS-2) (Gersting et al., 2008). While there is a growing understanding of the physiology of prostaglandin biosynthesis in the brain of the fetus, there is little known about the developmental changes in expression of PGHS isoforms and developmental changes in the local concentrations of PGE₂ that might be related to the now well-characterized developmental changes in autonomic reflex sensitivity, developmental changes in fetal hypothalamus-pituitary-adrenal axis activity, mechanisms controlling parturition in the sheep, or a host of other changes in physiological control mechanisms thought to be related to prostaglandin biosynthesis. The present study was performed to understand the ontogeny of expression of both PGHS isoforms and local PGE₂ concentrations in fetal brain regions. We performed the present experiments to test the hypothesis that there would be increases in the biosynthetic capacity for PGE₂ in one or more brain regions at the end of gestation, when readiness for birth is optimal.

1.1 Regional Comparisons

PGHS-1 and -2 mRNA were detected in every tissue tested at every gestational age tested, with mRNA abundance for each enzyme showing statistically significant ($p < 0.00001$) main effects of age and region in two-way ANOVA's. Evaluation of PGHS-1 mRNA expression across different brain regions revealed that expression was significantly ($p < 0.05$) greater in hippocampus and cortex than in cerebellum, in turn significantly ($p < 0.05$) greater than in pituitary and hypothalamus, significantly ($p < 0.05$) greater than in brainstem (Figure 1, bottom left panel). PGHS-2 mRNA expression by region was greatest in hippocampus, significantly ($p < 0.05$) lower in cortex, significantly ($p < 0.05$) lower in cerebellum, lower ($p < 0.05$) in hypothalamus, lower still in brainstem ($p < 0.05$), and lowest in pituitary ($p < 0.05$) (Figure 1, bottom right panel). With the exception of pituitary and hypothalamus PGHS-1 and -2 protein was detected in every brain region at every age. Because of the small mass of tissue in hypothalamus and pituitary, combined with the low levels of expression, we were unable to reliably detect PGHS-1 and -2 protein in these tissues.

Prostaglandin E₂ (PGE₂) was measurable in brainstem, cerebellum, hippocampus, and cerebral cortex (Figure 1, top panel). Again, tissue mass of hypothalamus and pituitary were insufficient to allow reliable extraction and measurement of PGE₂ in these tissues. The concentrations of PGE₂ were highest in cerebral cortex and hippocampus, lower in the cerebellum, and lower yet in the brainstem.

Tissue concentrations of PGE₂ and of PGHS-1 and -2 mRNA were greatest in the cerebral cortex compared to other brain regions (Figure 1). Concentrations of PGE₂ increased significantly with a peak concentration achieved at 145 DGA and 1 day postpartum (Figure 2, top panel; $p = 0.002$). PGHS-1 mRNA (Figure 2, middle left) decreased significantly ($p = 0.006$), reaching a nadir at 145 DGA and in adult sheep with a secondary transient increase in 7 d lambs. PGHS-1 protein (Figure 2, bottom left) significantly ($p < 0.001$) oscillated with developmental age. PGHS-1 protein was progressively increased, with the exception of a transient decrease near term (145 DGA). PGHS-2 mRNA expression (Figure 2, middle right) increased significantly ($p < 0.001$ by ANOVA) in late gestation and postnatally, reaching peak levels in lambs relative to 80 DGA. Apparent changes in PGHS-2 protein were not statistically significant ($p = 0.08$).

Tissue concentrations of PGE₂ in the hippocampus increased significantly after 80 DGA, reaching a plateau concentration at 120 DGA. The concentration of PGE₂ decreased in adult sheep relative to the 7 day lambs (Figure 3). PGHS-1 and -2 mRNA abundance in hippocampus were significantly varied at the different developmental ages ($p < 0.001$ and $p < 0.01$,

respectively, by ANOVA). Hippocampal PGHS-1 mRNA decreased significantly ($p < 0.001$) in late gestation fetal sheep and postpartum ages compared to 80 DGA fetuses (Figure 3, middle left). In contrast, PGHS-2 mRNA step-increased at 100 DGA, stayed constant between 100 DGA and 1 d postnatal, increased at 7 d postnatal, then returned back towards gestational levels in the adults (Figure 3, middle right, $p < 0.01$). PGHS-1 and -2 protein both significantly increased after 100 DGA ($p < 0.0001$ and $p < 0.001$, respectively), reaching peak concentrations between 130 DGA and 1 day postnatally for PGHS-1 and between 120 DGA and 7 days postnatally for PGHS-2 (Figure 3, lower panels). Abundance of both enzymes in hippocampus was significantly decreased in adult sheep.

Brainstem concentrations of PGE_2 were low throughout gestation relative to cortex and hippocampus and were unchanged (Figure 4). Analysis of both PGHS-1 and PGHS-2 mRNA abundance by ANOVA revealed significant variation with respect to developmental age ($p < 0.001$ and $p = 0.01$, respectively by ANOVA). Patterns of PGHS-1 and -2 mRNA abundance were similar, both low in fetal and neonatal life, and increased in the adult brainstem (Figure 4). Indeed, the overall statistical significance in PGHS-1 and -2 mRNA abundance was entirely accounted for by the changes in abundance of both transcripts in the adult brainstem ($p = \text{NS}$ by ANOVA for both PGHS-1 and -2 when this group was excluded from analysis). PGHS-1 protein decreased significantly as a function of age, and PGHS-2 protein increased significantly ($p < 0.001$ for both by ANOVA), reaching an apparent peak of expression at 1 day postnatal.

PGE_2 concentrations in cerebellum decreased significantly after 80 DGA (Figure 5). Analysis by ANOVA revealed statistically significant changes in PGHS-1 and -2 mRNA in cerebellum with respect to developmental age ($p < 0.05$ by ANOVA; Figure 5, middle panels). The expression patterns for both PGHS-1 and -2 were similar in that both were low before 120 days, relatively high between 120 and 145 DGA and lower after 145 DGA. PGHS-2 expression decreased significantly at 130 DGA relative to both 120 and 145 DGA (Figure 5, middle panel). Expression of PGHS-1 protein, also varying significantly in the different age groups ($p < 0.01$ by ANOVA), was essentially flat across the ages, with significant transient decreases at 100 DGA, and a more sustained decrease in 7 day postnatal lambs and adult sheep. (Figure 5, lower left). The pattern of PGHS-2 protein expression was also relatively flat, except for a transient decrease in abundance at 100 DGA compared to 130 DGA and older ($p < 0.001$, Figure 5, lower right).

In hypothalamus, both PGHS-1 and PGHS-2 expression at the mRNA ($p = 0.001$ for both by ANOVA) level varied significantly at different developmental ages (Figure 6, top panels). The patterns of hypothalamic PGHS-1 and -2 mRNA expression were generally similar. Expression of both genes was increased significantly after 100 DGA. PGHS-2 expression was relatively constant from 120 DGA onwards. PGHS-1 expression was significantly decreased at 145 DGA and 1 day postnatal relative to 120 and 130 DGA, giving the appearance of two peaks of expression, one at approximately 120 DGA and another postnatally.

Analysis of pituitary levels of PGHS-1 mRNA by ANOVA revealed significant variation in abundance amongst the different developmental ages ($p < 0.05$ for PGHS1, $p = \text{NS}$ for PGHS-2, Figure 6, bottom panels). PGHS-1 mRNA (Figure 6, bottom left) showed a step-increase at 120 DGA, a step decrease at 145 DGA, with relatively unchanged expression thereafter.

Stepwise linear regression of the logarithm of tissue concentration of PGE_2 , as measured in cortex, hippocampus, cerebellum, and brainstem, revealed a statistically significant correlation to the mRNA abundance (value of C_I) of PGHS-2 ($R^2 = 0.48$, $p < 0.0001$, $n = 116$), but not to PGHS-1 ($R^2 = 0.043$, $p > 0.5$) with the best-fit equation:

$$\log(\text{PGE}_2) = (2.577 \pm 0.084) - (0.09 \pm 0.008) \text{Ct}_{\text{PGHS2}}$$

Tissue concentrations of PGE_2 were not significantly correlated to the optical densities of the PGHS-1 and PGHS-2 protein abundances ($R^2=0.06$, $0.10 > p > 0.05$).

The results of this study provide evidence of significant developmental changes in the expression of both PGHS-1 and -2, in multiple regions of the fetal and postnatal brain. These data complement other studies detailing the expression in specific neuronal populations at specific ages (Breder et al., 1992; Norton et al., 1996; Thore et al., 1998; Tong et al., 2002). The relative abundance of PGHS-1 and -2 mRNA and PGE_2 tissue concentrations were highest in hippocampus and cerebral cortex. The present study suggests increasing biosynthesis of prostaglandins between 80 and 120 DGA, in hippocampus and cerebral cortex. Extensive neuronal proliferation and synapse formation mark this period of gestation (Nelson et al., 1995; Makarenko et al., 2005; Cudd, 2005). Metabolites of arachidonic acid have been found to control filopodial behavior in neurons (Geddis et al., 2004). It is therefore possible that the dramatic upregulation of PGHS expression in cortical regions during the latter half of gestation may be involved in neuronal guidance and synaptogenesis during this period of gestation. It is also possible that PGHS-2 mediated prostaglandin synthesis may be involved in cortical activity. Functional studies in adult humans have demonstrated blockade of cortical activity after administration of PGHS-2 inhibitors (Baliki et al., 2005). Recent electrophysiological evidence has demonstrated that inhibition of PGHS-2, but not PGHS-1, regulates PGE_2 -based hippocampal long term plasticity (Chen et al., 2002).

The abundant expression of PGHS-2 mRNA, the upregulation of PGHS-2 mRNA and the contrast with the downregulation of PGHS-1 mRNA in hippocampus and cerebral cortex was not mirrored in other brain regions. It is possible that the upregulation of PGHS-2 mRNA abundance in hippocampus is related to the changing endocrine and/or physiological environment of the fetus. A likely stimulus to PGHS-2 mRNA expression in late gestation is increased estrogen action, secondary to increased fetal plasma estrogen concentrations in late gestation (Yu et al., 1983; Nathanielsz et al., 1982). We have reported that estradiol increases fetal CNS PGHS-2 expression (Wood and Giroux, 2003), and we have recently found that blockade of fetal CNS estrogen receptors reduces PGHS-2 expression (Schaub et al., 2008). It is possible, however, that there are other stimuli. PGHS-2 expression is induced in response to reduced cerebral blood flow (Purinton and Wood, 2002; Tong et al., 2002; Wood and Giroux, 2003). Because none of the ewes in this study were in active labor, it seems unlikely that uterine activity altered the flow of blood and/or oxygen to the fetuses.

Metabolites of PGHS, such as PGE_2 , generated within the fetal brain are known to stimulate the neuroendocrine mechanisms controlling fetal hypothalamus-pituitary-adrenal axis activity (Tong et al., 1998; Gersting et al., 2008). The specific neural substrate for this influence of prostanoids on fetal ACTH secretion is not known. However, we do know that chronic blockade of PGHS-2 enzymatic activity prevents the preparturient rise in fetal ACTH secretion that heralds spontaneous parturition (Gersting et al., 2008).

We measured tissue concentrations of PGE_2 as one index of PGHS activity. While PGE_2 is only one of the downstream products of PGHS activity, the high concentrations measured in cerebral cortex and hippocampus are consistent with the abundant mRNA for PGHS-1 and -2 in these regions. The patterns of PGE_2 concentration in both cerebral cortex and hippocampus mirror the changes in mRNA for PGHS-2, perhaps suggesting that PGHS-2 activity is important for PGE_2 biosynthesis in these tissues. Supporting this notion is a recent observation from our laboratory that infusion of nimesulide, a specific inhibitor of PGHS-2 activity, into

the lateral cerebral ventricle of the fetal sheep, reduces tissue PGE₂ concentrations in cerebral cortex, hippocampus, hypothalamus, cerebellum, and brainstem (Gersting et al., 2008).

The apparent association of tissue concentrations of PGE₂ with PGHS-2 expression in hippocampus and cerebral cortex was observed in a more global analysis of PGHS expression and tissue PGE₂ concentration. Multiple regression analysis revealed that the tissue PGE₂ was strongly correlated with PGHS-2 mRNA, more so than with PGHS-1 mRNA. Interestingly, the tissue PGE₂ did not correlate with the protein abundance. Protein and mRNA turnover rates for PGHS-1 and -2 are controlled independently (Gibson et al., 2005). For example, PGHS-1 and -2 undergo “suicide inactivation”, where enzymatic activity results in self-inactivation (Smith and Lands, 1972; Mbonye et al., 2008). Suicide inactivation results in shifts in the molecular weight or destruction of the immunoreactivity of the enzyme at native molecular weight that, in turn, result in an apparent discordance between mRNA and protein abundance (Orning et al., 1992). Suicide inactivation could lead to reciprocal changes between mRNA and protein for either or both of these enzymes. Supporting this interpretation of the data is our observation that intracerebroventricular infusion of nimesulide (a specific PGHS-2 inhibitor) increased PGHS-2 protein and decreased PGHS-2 mRNA in all of the brain regions investigated in the present study. Those previous data suggested that inhibition of the enzyme stabilized the protein and reduced its turnover rate, resulting in a reduction in the mRNA encoding the protein (Gersting et al., 2008). Changes in subcellular distribution can also contribute to this discordance. PGHS-1 and -2 are expressed on the endoplasmic reticulum and nuclear envelope of endothelial cells, as well as in the peri-nuclear region and axons of neurons (Spencer et al., 1998; Norton et al., 1996). This may indicate that some fraction of translated protein migrates away from the site of mRNA synthesis to distant brain regions via axonal transport. Discrepancies between mRNA and protein abundance can also be confounded by changes in PGHS mobility resulting from tyrosine phosphorylation or other posttranslational processing (Parfenova et al., 1998).

In conclusion, the current study has demonstrated that PGHS-1 and -2 are expressed in the ovine fetal brain throughout the latter half of gestation, and that the patterns of expression are region-specific. A high level of expression of both enzymes and high tissue concentrations of PGE₂ in the cerebral cortex and hippocampus suggests the possibility that prostanoid synthesis in these brain regions might play an important role in brain development and function in the fetus as it nears parturition. It is possible that the biosynthesis of prostaglandins in the fetal brain organize various aspects of maturation, including synaptogenesis, facilitation of specific reflex pathways, and overarching physiological processes, such as the blood pressure regulation or the timing of parturition. We suggest that, because prostaglandin biosynthetic enzymes appear to be transiently upregulated at specific times in specific brain regions, blockade of these enzymes during the latter half of gestation is likely to alter the timecourse or perhaps the final outcome of fetal brain development. We therefore conclude that prostaglandin signaling in the developing brain is likely to be of fundamental importance for both brain development and for regulation of physiological processes by the brain.

2. MATERIALS AND METHODS

Animals and Collection of tissues

The experiments were approved by the University of Florida Animal Care and Use Committee and were performed in accordance with the Guiding Principles for Use of Animals of the American Physiological Society.

Timed-dated (80, 100, 120, 130, or 145 days gestational age (DGA), n=4–5 per group, term=148 days) pregnant ewes, not in labor, carrying singletons or twins (one per group) were sacrificed with 20 ml Euthasol® solution (7.8 g pentobarbital and 1g phenytoin sodium; Virbac

AH, Inc; Fort Worth, TX) administered intravenously. After removal of the fetus from the uterus, fetal cortex, cerebellum, hippocampus, hypothalamus, medullary brainstem, and pituitary were isolated and immediately frozen in liquid nitrogen and stored at -80°C . One day and one week postpartum animals, as well as 1-month post-delivery ewes were euthanized directly and tissues collected as above. We collected hypothalamus, pituitary, hippocampus, and medullary brainstem because these tissues are central to the control of fetal hypothalamus-pituitary-adrenal axis activity, responsiveness to fetal stress, and control of parturition in the sheep. We chose all of the brain regions for study because alterations in the fetal environment have been shown to alter the expression of either PGHS-1, PGHS-2, or both enzymes (Tong and Wood, 1998; Tong et al., 2002; Wood and Giroux, 2003; Schaub et al., 2008). The medullary brainstem tissue was sectioned ~ 1 mm rostral to the obex and at the caudal medulla-rostral spinal cord border. Frontal cortex consisted of the most rostral 1 cm block of prefrontal cortex. Whole hippocampus was dissected bilaterally. Whole cerebellum was detached from brainstem after sectioning the cerebellar peduncles. Pituitary was removed in its entirety after removal of the diaphragma sella. Hypothalamus was removed as a single block of tissue, bounded on the rostral edge by the rostral edge of the optic chiasm, on the caudal side by the caudal edge of the median eminence, and on the sides by the edges of the median eminence. Entire pituitaries and hypothalami were processed for mRNA extraction, while other areas were divided for simultaneous mRNA and protein analysis.

RNA isolation and Real-time RT-PCR

Total RNA was isolated from tissues using Trizol® reagent (Invitrogen, Carlsbad, CA) according to manufacturer's directions. Total RNA was treated with DNase (Ambion DNA-free™; Ambion, Inc, Austin, TX: 2U/10 μg RNA) and RNA concentration was subsequently determined by spectrophotometry.

For each sample, 2 μg of total RNA was reverse transcribed using the High Capacity cDNA kit (Applied Biosystems, Foster City, CA), according to manufacturer's instructions. The resulting cDNA was used in real-time PCR reactions for PGHS-1 or -2 (100ng), and 18S ribosomal RNA (0.1ng) using TaqMan® Universal PCR Master Mix according to manufacturer's instructions (Applied Biosystems, Foster City, CA) and probes/primers as previously described (Wood and Giroux, 2003). All reactions were run in triplicate in optical grade 96-well plates and caps on a ABI 7000 thermocycler (Applied Biosystems, Foster City, CA). Relative expression levels were calculated by determining the difference in cycle number (ΔC_t) between the PGHS-1 or PGHS-2 samples and the corresponding ribosomal RNA samples. Mean ΔC_t 's were calculated in triplicate, and for all samples contained in a single sample age group. ΔC_t values were adjusted by subtracting the reference value of the 80-day samples, resulting in a $\Delta\Delta C_t$ value. Fold change expression was calculated by using $2^{-\Delta\Delta C_t}$ to normalize numbers to the 80-day age group (Livak and Schmittgen, 2001).

Protein Isolation and western blotting

Samples were homogenized in Potter-Elvehjem glass/Teflon tissue grinders (Wheaton, Millville, NJ, USA) with 5 mL of ice cold microsomal homogenization buffer (1mM EDTA, 0.32 M sucrose, 0.1 mM dTT, 1mM HEPES, pH 7.4) using a motor drive (Dynamix, Fisher Scientific, Pittsburgh, PA, USA) for 10–15 strokes set at speed #5. Twenty microliters of 200 mM phenylmethylsulfonylfluoride (PMSF) was added and homogenates were centrifuged for ten minutes at $550 \times g$ at 4°C . For brainstem samples, the resulting supernatants were centrifuged for twenty minutes at $20,000 \times g$ at 4°C . To isolate microsomes, the second supernatant was collected and centrifuged at $100,000 \times g$ for one hour at 4°C . The resulting pellet was resuspended in 150–200 μL ice cold homogenization buffer. Protein concentration was quantified using the method of Bradford (Bradford, 1976).

Western blot analysis was performed, as described previously, by separating 10–40 µg of each sample in a 7.5% Tris-HCl SDS-polyacrylamide gel (BioRad, Hercules, CA) and transferred overnight onto nitrocellulose membranes (BioRad) (Wood and Giroux, 2003). Membranes were immunostained using antibodies for PGHS-1 and -2 (PGHS-1: rabbit anti-ovine PGHS-1 at 1:2500, #PG-16, Oxford Biomedical Research, Oxford, MI; PGHS-2: mouse anti-ovine PGHS-2 at 1:1000, #160112, Cayman Chemical, Ann Arbor, MI) diluted in 2 or 3% non-fat dry milk in Tris buffered saline with 0.1% Tween-20 (PBST), respectively. Staining was visualized using peroxidase linked secondary antibodies (PGHS-1: donkey anti-rabbit at 1:3000; PGHS-2: sheep anti-mouse at 1:3000, #'s NA934 and NA931, respectively, Amersham Biosciences, Piscataway, NJ, USA) and enhanced chemiluminescence (ECL, Amersham, Arlington Heights, IL). Images were quantified using a ChemiDoc XRS System (Bio-Rad) and band intensities were quantified using Quantity One software (BioRad). Data are expressed as mean band densities $\pm$ SEM in arbitrary units.

Tissue concentrations of PGE₂

Tissue concentrations of PGE₂ were measured after extraction of prostanoids from 100 µL of protein homogenates using 8 vol ethyl acetate, which was then evaporated under nitrogen stream. The concentrations of PGE₂ were measured using a commercially-available ELISA (catalog number 514010, Cayman Chemical, Ann Arbor, MI). The mass of extracted PGE₂ was normalized to the mass of protein in the aliquot of homogenate extracted.

Statistics

mRNA abundance was analyzed as values of ΔC_t to avoid heteroscedacity produced by calculation of fold change ($2^{-\Delta\Delta C_t}$). Overall changes in mRNA abundance and differences among tissues were assessed using two-way ANOVA. Individual groups were compared using simple effects contrasts (Field, 2005). Overall changes in protein abundance were analyzed by three-way ANOVA, controlling for developmental age, brain region, and gel. Because each brain region ontogeny was analyzed using two separate gels, and because differences among regions would have been influenced by differences in exposure and development in the individual western blots, a higher order ANOVA was used to statistically control for the differences between gels as we have previously described (Saoud and Wood, 1996). Changes in mRNA or protein expression as a function of age were evaluated by two-way ANOVA (age and region) followed by simple effects contrasts at a significance level of $\alpha \leq 0.05$ (Winer, 1971; Field, 2005). Values of PGE₂ tissue concentration were logarithmically transformed where appropriate to reduce heteroscedacity prior to ANOVA. All tests were performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

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Figure 1.

Relative tissue concentration of PGE₂ (top panel) and mRNA abundance of PGHS-1 (bottom left panel) and PGHS-2 (bottom right panel) among brain regions measured at either 80 (black bars), 120 (light gray bars), or 145 (dark gray bars) DGA. Brain regions are abbreviated as bs=brainstem, cb= cerebellum, cx=cortex, hip=hippocampus, hypo=hypothalamus, pit=pituitary. Significant differences among brain regions (*i.e.*, *post hoc* comparison of regions without respect to specific age) are represented as different letters above groups of bars.

Figure 2.

Ontogenetic changes in tissue concentrations of PGE₂ (top panel), PGHS-1 and -2 mRNA (middle panels) and protein (bottom panels) in cerebral cortex. Data are shown as mean $\pm$ SEM. Representative western blots are superimposed on the graphs reporting summary protein data. Different letters next to individual mean values in each graph represent statistically significant differences.

Figure 3.

Ontogenetic changes in tissue concentrations of PGE₂ (top panel), PGHS-1 and -2 mRNA (middle panels) and protein (bottom panels) in hippocampus. Data are shown as mean $\pm$ SEM. Representative western blots are superimposed on the graphs reporting summary protein data. Different letters next to individual mean values in each graph represent statistically significant differences.

Figure 4. Ontogenetic changes in tissue concentrations of PGE₂ (top panel), PGHS-1 and -2 mRNA (middle panels) and protein (bottom panels) in brainstem. Data are shown as mean $\pm$ SEM. Representative western blots are superimposed on the graphs reporting summary protein data. Different letters next to individual mean values in each graph represent statistically significant differences.

Figure 5.

Ontogenetic changes in tissue concentrations of PGE₂ (top panel), PGHS-1 and -2 mRNA (middle panels) and protein (bottom panels) in cerebellum. Data are shown as mean $\pm$ SEM. Representative western blots are superimposed on the graphs reporting summary protein data. Different letters next to individual mean values in each graph represent statistically significant differences.

Figure 6.

Ontogenetic changes in PGHS-1 and -2 mRNA in hypothalamus (top panels) and pituitary (bottom panels). Data are shown as mean $\pm$ SEM. Different letters next to individual mean values in each graph represent statistically significant differences.