Prostaglandin G/H Synthase-2 Is a Major Contributor of Brain Prostaglandins in the Newborn*

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In order to understand the molecular basis of the elevated cerebral prostaglandin levels in the newborn, we compared the expression of the mRNAs and proteins of prostaglandin G/H synthases (PGHS), PGHS-1 and PGHS-2, in various regions of the brain and the microvasculature of newborn (1–2-day-old) and juvenile (4–7-week-old) pigs and also measured the relative contribution of PGHS-2 to cerebral prostaglandin synthesis both in vivo and in vitro by using a novel inhibitor of PGHS-2, NS-398. Ribonuclease protection assays using total RNA isolated from various regions of the porcine brain revealed that, unlike PGHS-1 mRNA, PGHS-2 mRNA was abundantly expressed in the cortex and the microvasculature of the newborn compared with those of the juvenile animal. PGHS-2 immunoreactive protein comprised the majority of total PGHS enzyme in neonatal cerebral microvasculature due to a 2–3-fold lower expression of immunoreactive PGHS-1 protein. Inhibition of PGHS-2 by NS-398 decreased the rate of prostaglandin synthesis by purified cerebral microvessels of the newborn by approximately 65% and of juvenile pigs by 30%. The decrease in brain tissue prostaglandin concentrations following intravenous administration of NS-398 was greater in newborn pigs (90%) than in the juvenile animals (30%). Furthermore, NS-398 substantially reduced the net in vivo cerebrovascular production of prostaglandins in newborn pigs. Taken together, these results indicate that PGHS-2 is the predominant form of prostaglandin G/H synthase in the newborn brain and cerebral microvasculature and the main contributor to the brain prostaglandin levels in the newborn animal.

Prostaglandins act as modulators in several neurological (1, 2) and cerebral hemodynamic functions (3, 4). During the perinatal period the concentrations of prostaglandins in blood and brain in the newborn are higher than those in the normal adult (5, 6). These higher levels of cerebral prostanoids in the newborn significantly affect cerebral blood flow autoregulation as well as cause down-regulation of prostanoid receptor expression and receptor function in brain (7, 8). However, the cause of increased prostaglandin levels and the relative contributions of the two prostaglandin G/H synthases (PGHS)* to prostanoid synthesis in the neonatal brain are not yet known.

Of the two prostaglandin G/H synthases so far described, PGHS-1 (EC 1.14.99.1) is constitutively expressed in all tissues, albeit to varying degrees (for reviews see Refs. 9–11). The other isozyme, PGHS-2, shares significant homology with PGHS-1 in amino acid sequence (11) and exhibits similar enzymatic properties (12–14) but differs in its pharmacological properties (14–16), mRNA size (11), chromosomal location (17), and gene organization (18, 19). Moreover, PGHS-2 can be rapidly induced in various tissues by diverse stimuli such as mitogenic agents, growth factors (20, 21), hormones (22, 23), inflammatory agents (24), synaptic activity (25), and muscle stretch/relaxation (26). Elevation of PGHS-2 expression in inflammation (10, 11) and suppression of PGHS-2 gene activity by dexamethasone and other corticosteroids both in tissue cultures (20) and in vivo (27) suggest that PGHS-2 may have a role in inflammatory response. Nonetheless, low but varying levels of PGHS-2 expression have been found in all tissues (28) by using the reverse transcription-polymerase chain reaction technique. Thus, despite its induction in inflammation and mitogenesis, the role of PGHS-2 under normal physiological conditions of increased prostaglandin synthesis in the brain, as seen in the perinatal period (5, 6), is still a matter of conjecture. This is of particular interest given that expression of the other isozyme, PGHS-1, has been shown to be low in the newborn and increases to reach maximum levels in the adult (29). We hypothesized that the elevated prostaglandin G/H synthase activity in the newborn brain could be due to increased expression of PGHS-2. For this purpose, we analyzed the expression of porcine PGHS-1 and PGHS-2 mRNAs by ribonuclease protection assays and analyzed the expression of PGHS-1 and PGHS-2 proteins by immunochemical methods in the newborn and the juvenile animals. We also examined the relative contribution of PGHS-2 to the cerebral production of prostaglandins both in vivo and in vitro by using a PGHS-2 inhibitor, NS-398.

EXPERIMENTAL PROCEDURES

Materials—Polyclonal rabbit antibodies specific to PGHS-1 and PGHS-2 were generously provided by Drs. A. W. Ford-Hutchinson and G. P. O’Neill (Merck Frosst Center for Therapeutic Research, Pointe Claire, Canada). Purified ovine PGHS-1 and PGHS-2 enzymes were purchased from Cayman Chemical (Ann Arbor, MI). [3H]CTP (3000 Ci/mmol), [3H]-PGE2 (191Ci/mmol), [3H]-PGF2α (219 Ci/mmol), [3H]-6-keto-PGF1α (175 Ci/mmol), and enhanced chemiluminescence kit were purchased from Amersham Corp. Radiomunnoassay kits for PGE2, PGF2α, and 6-keto-PGF1α were from Advanced Magnetics (Boston, MA).

The abbreviations used are: PGHS, prostaglandin G/H synthase(s); PIPES, 1,4-piperazineethanesulfonic acid; PGE, prostaglandin E; PGF, prostaglandin F.
MA); Aprotinin and leupetin were from Boehringer Mannheim Canada (Montreal, Canada). Soybean trypsin inhibitor (type II-S), phenylmethylsulfonyl fluoride, arachidonic acid, ibuprofen, indomethacin, phosphoribulose 1-mutase, dimethylsulfoxide, and β-mercaptoethanol were from Sigma. NS-398 was from Biomol (Plymouth Meeting, CA). Ficoll-400, ribonuclease A, diiodo-UTP, and T a sequencing kit were from Amersham (UK). PGEM-3 plasmid DNA sequencing kit was from Promega (Madison, WI). Protein assay and electrophoretic reagents were purchased from Bio-Rad. Taq polymerase, deoxynucleotides, guanidinium isothiocyanate, Tα DNA ligase, Taq polydeoxi nucleotide kinase, immunoprecipitation, M-MLV reverse transcriptase, random hexamers, and restriction enzymes were purchased from Promega (Madison, WI). All other chemicals were of analytical reagent grade and were purchased from either Sigma or ICN Biochemicals.

Animals—Newborn (1-2-day-old) and juvenile (4-7-week-old) pigs were purchased from Fermen Ménard Inc, L’Ange Gardien, Quebec and used according to a protocol of the Animal Care Committee of Ste-Justine Hospital, Montreal.

Purification of Porcine Cerebral Microvessels—Newborn and juvenile pigs were anesthetized with 2% halothane and killed by intracardiac injection of pentobarbitol (120 mg/kg). The brains were perfused with heparinized saline (200–250 ml for newborn and 1 liter for juvenile pigs) to eliminate blood elements. The brain was removed and immediately kept in ice-cold Krebs buffer (120 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl2, 1.25 mM MgCl2, 10 mM d-glucose). Brain tissue was homogenized gently (5–6 strokes) in ice-cold phosphate-buffered saline containing 20% Ficoll-400 using a glass homogenizer with loose fitting glass pestle. The homogenate was centrifuged at 20,000 × g for 20 min at 4°C. The pellet containing the microvessels was washed 3 or 4 times with 20 volumes of ice-cold phosphate-buffered saline to eliminate Ficoll. The resultant microvesSEL preparations were assessed for purity by light microscopy and γ-gutamyl transpeptidase activity (8, 30). The microvessels were used immediately for either total RNA isolation or prostaglandin G/H synthase assays.

Isolation of cDNA Probes for Porcine PGHS-1 and PGHS-2—Total and poly(A)1 RNA from porcine ileum and cerebral microvascular smooth muscle cell cultures that were stimulated with phorbol 12-myristate (100 ng/ml) were obtained as described previously (31). Two micrograms of poly(A1 RNA was reverse transcribed using 400 units of Moloney murine leukemia virus reverse transcriptase and 10 μg random hexamers, in a 50-μl reaction containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, and 0.5 μg each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. An aliquot of the cDNA (equivalent to 1 μg of poly(A1 RNA) was amplified using 1.5 units of Taq DNA polymerase in a 100-μl reaction buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM each of dCTP, dGTP, dATP, and dTTP, for 1 h at 42°C. After the surgery, halothane was discontinued, and the animals were ventilated with air using a Harvard animal respirator. Catheterization of the animals was performed as described previously (4). The animals were allowed to stabilize for 2 h and then were injected with 0.15% halothane. Tracheostomy was performed, and the animals were ventilated with air using a Harvard animal respirator. Catheterization of the right femoral artery for measurement of blood pressure (Gould TA240), blood gases, and arterial blood prostaglandin G/H synthase levels were determined by radioimmunoassay as described previously (39).

Tissue Prostaglandin Levels and Cerebrovascular Prostaglandin Synthesis—Prostaglandins were synthesized in vivo—Newborn and juvenile pigs were anesthetized with medullia oblongata, hippocampus, thalamus, periventricular area, retina, choroid, lung, and cerebral microvasculature of newborn and juvenile pigs as described above. Aliquots of the total RNA were resolved by formaldehyde-agarose gel electrophoresis and stained with ethidium bromide to assess the integrity and also to verify quantitation by spectrophotometry. The RNAase protection assays were conducted following a published protocol (37) with minor modifications. Briefly, 50–100 μg of total RNA was incubated overnight at 50°C with 5 × 106 cpm of either PGHS-1 or PGHS-2 probes in 40 μl of hybridization buffer (80% deonized formamide, 40 mM PIPEs, pH 6.8, 1 mM EDTA, and 0.4 μg NaCl). The RNA hybrids were digested in 400 μl of digestion buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 0.3 μg NaCl) containing ribonuclease A (40 μg/ml) for 30 min. The digestion products were purified by phenol/chloroform extraction and ethanol precipitation. The protected RNA fragments were resolved on a 6% polyacrylamide gel and autoradiographed using Kodak-X-Omat film. In addition, the bands were visualized and quantified by Phosphorimage (Molecular Dynamics).

Immunoprecipitation and Western Blotting of PGHS-1 and PGHS-2—Purified microvessels from newborn and juvenile brains were homogenized in ice-cold buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, 10 μg/mL each of leupeptin, aprotinin, soybean trypsin inhibitor, 0.2 mM phenylmethylsulfonyl fluoride) with an Omni 2000 tissue grinder (Omnin International, Waterbury, CT) for 15–20 s. The homogenates were centrifuged at 12,000 × g for 10 min at 4°C. Total protein concentration of the supernatant was determined by using the Bradford dye-binding assay (Ref. 38; Bio-Rad protein assay reagent) and bovine serum albumin as the standard. All operations, unless specified, were conducted at 4°C. An aliquot of the total protein (2 mg) was preadsorbed with 50 μl of immunoprecipitin for 30 min, followed by centrifugation at 12,000 × g for 10 min to remove the immunoprecipitin. The supernatants were incubated with PGHS-1- or PGHS-2-specific antibodies for 1.5 h with gentle agitation, and the immune complexes were collected by incubation with 50 μl of immunoprecipitin for 1 h. The antibodies for PGHS-1 and PGHS-2 were assured by the supplier (Merk-Frast) and tested by us both in immunoprecipitation and Western blotting. The immune precipitates were washed with wash buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) three times, denatured in SDS-sample buffer (125 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 30% (v/v) glycerol, 0.1 mg/ml Bromophenol Blue) for 15 min at room temperature, and centrifuged at 12,000 × g for 15 min to remove the immunoprecipitin. After adding β-mercaptoethanol to a final volume of 10%, the supernatants were boiled for 5 min before loading on SDS-polyacrylamide gels. The proteins were electrophoretically transferred to nitrocellulose membrane, and the non-specific binding sites on the membranes were blocked with buffer A (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% (v/v) Tween 20) containing 3% skim milk for 1 h. The membranes were briefly rinsed with buffer B (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 150 mM NaCl) and incubated for 1 h in buffer A containing 1% skim milk and PGHS-1- or PGHS-2-specific polyclonal rabbit antibodies (1:7500). The membranes were washed six times (5 min each) with buffer B. A second incubation with horseradish peroxidase-conjugated anti-rabbit IgG antibodies (Amersham Corp.) in buffer A containing 1% skim milk for 1 h, followed by several washes of the membranes was conducted as described above. Finally, the immunoreactive bands were visualized by using the enhanced chemiluminescence kit (Amersham Corp.) as instructed by the manufacturer.
with either saline or NS-398 (10 mg/kg intravenously). Forty-five min later, the animals were killed by intracardiac injection of pentobarbital (120 mg/kg), and the brain and eyes were immediately removed and frozen in liquid nitrogen. Brain cortex and retinal prostaglandin concentrations were determined by radioimmunoassay after extraction on octadeylsilica silica columns (40).

In a separate study designed to measure cerebrovascular production of prostaglandins, newborn pigs were anesthetized as above, and catheters were placed in the left ventricle via the right subclavian artery and in the sagittal sinus. After the animals were allowed to stabilize for 2 h, blood samples were collected from the femoral artery and sagittal sinus, before and 45 min after injection of NS-398 (10 mg/kg intravenously) for prostaglandin determination (41). Cerebral blood flow was measured using radiolabeled microspheres as described in detail previously (4, 41). The net cerebrovascular production of prostaglandins was calculated as total cerebral blood flow × difference in prostaglandin concentrations of sagittal sinus and arterial blood samples and expressed as ng/min/100 g of brain tissue. Similar studies were not done on juvenile pigs because of surgical difficulties in catheterization of sagittal sinus.

Statistical Analysis—Data were analyzed by paired or unpaired Student's t test, and a p < 0.05 was assumed to denote significance. Throughout this paper data are presented as means ± S.E.

RESULTS

Tissue Distribution of PGHS-1 and PGHS-2—The cDNA probes of PGHS-1 (0.82 kilobase) and PGHS-2 (0.8 kilobase) isolated by reverse transcription-polymerase chain reaction of the mRNA comprised approximately 45% of the reading frames of both cDNAs. Sequence analysis (data not shown) revealed that both porcine enzymes shared significant amino acid identity (85–90%) with their respective murine (32, 34), human (34, 35), and rat (36) enzymes; in addition, some functionally and catalytically important amino acids as well as amino acid sequences identified in porcine PGHS-1 and PGHS-2 cDNAs were totally conserved. Radiolabeled cDNA probes generated from these cDNAs were used in RNase protection assays of total RNAs isolated from various tissues of the newborn and juvenile pigs. As shown in Fig. 1A and B, PGHS-1 mRNA was expressed in all tissues at varying levels. Non-neural tissues such as the choroid and lungs of both newborn and juvenile animals (lanes 16–19) expressed PGHS-1 mRNA abundantly. Among tissues of neural origin, high expression of PGHS-1 was seen in the medulla oblongata (lanes 6 and 7). Densitometric analyses of the radioactive bands revealed that there was 1.5–2.0-fold higher expression of PGHS-1 in brain tissue of the juvenile than in that of the newborn animal (Fig. 1B), especially in the cortex (lanes 2 and 3), cerebelum (lanes 4 and 5), medulla oblongata (lanes 6 and 7), hippocampus (lanes 10 and 11), and thalamus (lanes 12 and 13). In contrast to PGHS-1 mRNA, PGHS-2 mRNA in both neural and non-neural tissues was barely detectable; a significant exception was the brain cortex and, to a lesser extent, the choroid of the newborn (Fig. 1, A and B, lanes 3 and 17, respectively). Newborn brain cortex contained a 3-fold greater expression of PGHS-2 mRNA compared with juvenile cortex (Fig. 1, A and B, lanes 2 and 3). The abundance of PGHS-2 mRNA was variable in different regions of the brain and in other tissues; where PGHS-2 mRNA was detectable, expression of PGHS-2 was higher in the newborn than in juvenile animals.

PGHS-1 and PGHS-2 mRNA Levels in the Brain Cortex and Microvasculature—The results of PGHS-1 and PGHS-2 mRNA expression in cerebral cortex and microvasculature of newborn and juvenile pigs analyzed by ribonuclease protection assay are presented in Fig. 2A, PGHS-1 mRNA (Fig. 2A) in the newborn cortex (lane 3) and the microvessels (lane 5) was lower than that in the juvenile cortex (lanes 2) and microvessels (lane 4), as revealed by densitometric analysis (newborn/juvenile ratio: 0.63–0.77; Fig. 2C). In contrast, PGHS-2 mRNA (Fig. 2B) was more abundant in newborn tissues (lanes 3 and 5) than in adult tissues (lanes 2 and 4) (newborn/juvenile ratio: 2.0–2.4; Fig. 2C).

PGHS-1 and PGHS-2 Protein Expression in Cerebral Microvasculature—To demonstrate if the ontogenic differences in the mRNA levels of PGHS-1 and PGHS-2 in newborn and juvenile cerebral microvasculature were reflected in the protein levels, immunoprecipitations of PGHS-1 and PGHS-2 proteins with specific antibodies followed by Western blotting were conducted (Fig. 3). There was a 3–4-fold lesser expression of immunoreactive PGHS-1 in newborn than in juvenile cerebral microvasculature, whereas expression of PGHS-2 was comparable in newborn and juvenile tissues.

Ontogenic Differences in Prostanoid Synthesis by Purified Cerebral Microvasculature—The rates of prostaglandin synthesis by homogenates of cerebral microvessels from newborn and juvenile pigs in the presence of 50 μM arachidonic acid were compared. Prostaglandins were synthesized at apparent pCO2, blood pressure, and heart rate of the animals. PGE2 and

![Image](https://example.com/image.png)
Prostaglandin G/H Synthase-2 Expression in Neonatal Brain

PGHS-2 was not detectable (Fig. 1, juvenile animals only by 26–30%. In retina where mRNA for contrast, NS-398, decreased brain prostaglandin levels in the cortex of the newborn (Fig. 5), comparable with what was reduced prostaglandin concentrations by retina of the newborn than of juvenile pigs (Fig. 5). NS-398 caused a 21–33% reduction in PGE₂ and PGF₂α activity and, in turn, high prostaglandin levels in newborn. Moreover, PGHS-2 comprised the majority of immunoreactive PGHS proteins in the newborn brain cortex. Second, NS-398, a relatively specific PGHS-2 inhibitor, produced a much greater decrease in prostaglandin synthesis in the newborn compared with the juvenile pig.

Fig. 2. mRNA levels of PGHS-1 and PGHS-2 in brain cortex and microvasculature from newborn and juvenile pigs. A, PGHS-1; B, PGHS-2. In both panels: lane 1, tRNA (control); lane 2, juvenile brain cortex; lane 3, newborn brain cortex; lane 4, juvenile cerebral vasculature; lane 5, newborn cerebral vasculature; lane 6, antisense RNA probe. The antisense RNA probe for PGHS-1 was 293 nucleotides, and for PGHS-2 it was 209 nucleotides; the protected fragments were 235 and 185 nucleotides for PGHS-1 and PGHS-2, respectively. Total RNA (100 µg) was subjected to RNase protection assay as described under “Experimental Procedures.” Autoradiographic exposure was for 6 days. C, newborn/juvenile ratio of mRNA for PGHS-1 and PGHS-2 in cerebral cortex and microvasculature.

PGF₂α concentrations were higher in the brain cortex and retina of the newborn than of juvenile pigs (Fig. 5). NS-398 reduced prostaglandin concentrations by ≥90% in the brain cortex of the newborn (Fig. 5), comparable with what was observed previously using ibuprofen and indomethacin (42); in contrast, NS-398, decreased brain prostaglandin levels in the juvenile animals only by 26–30%. In retina where mRNA for PGHS-2 was not detectable (Fig. 1, lanes 16 and 17), NS-398 caused a 21–33% reduction in PGE₂ and PGF₂α concentrations in tissues from newborn and juvenile animals.

To assess the effect of NS-398 on cerebrovascular prostaglandins in the newborn animal, their concentrations in blood samples from arterial and sagittal sinus blood were determined before and after the injection of NS-398 to newborn pigs, and in vivo cerebrovascular prostaglandin production was calculated (4). Net cerebrovascular production of prostaglandins was reduced by >65% in response to NS-398 treatment (Fig. 6); this decrease was unrelated to cerebral blood flow, which actually increased by 33–45% after NS-398 treatment.

Fig. 3. Immunoblot of PGHS-1 and PGHS-2 proteins in newborn and juvenile pig cerebral microvasculature. Lane 1, juvenile; lane 2, newborn; lane 3, 50 ng of purified ovine PGHS-1 or PGHS-2 protein (Cayman). Arrows point to PGHS-1 and PGHS-2 polypeptides (70 kDa) that were immunoprecipitated with specific antibodies from the detergent-lysates of cerebral microvasculature (2 mg of protein) isolated from newborn and juvenile pig brains. Following electrophoretic transfer of the proteins to nitrocellulose membranes, PGHS-1 and PGHS-2 polypeptides were detected by Western blotting and visualized by enhanced chemiluminescence (Amersham Corp.).

DISCUSSION

Several studies have reported that prostaglandin levels are elevated in the neonatal blood and brain during the perinatal period (5, 6, 8). However, the reasons for this increase in cerebral prostaglandins are not known. We tested the hypothesis that high prostaglandin levels in brain during neonatal period may be due to increased PGHS-2 activity and provided two main lines of evidence in support of this hypothesis. First, brain cortex and microvasculature of the newborn expressed more PGHS-2 mRNA than the juvenile animal, whereas PGHS-1 mRNA was more abundant in the juvenile than in cerebral cortex and microvessels of the newborn. Moreover, PGHS-2 comprised the majority of immunoreactive PGHS proteins in the newborn brain cortex. Second, NS-398, a relatively specific PGHS-2 inhibitor, produced a much greater decrease in prostaglandin synthesis in the newborn compared with the juvenile pig.

RNA protection assays revealed that PGHS-1 mRNA was ubiquitously expressed but that its abundance differed within various regions of the brains of the newborn and juvenile pigs. The hindbrain and midbrain contained highest expression of PGHS-1. Similar observations have been made by others using different techniques such as Northern analysis and in situ hybridization (29, 43). However, the expression of PGHS-1 in brain is considerably lower than that in peripheral tissues such as the choroid and lungs, in accordance with other data (28, 29). In addition to its diverse tissue expression, PGHS-1 mRNA increased with age in brain (29) and cerebral vasculature (Fig. 2); in other tissues such as fetal cotyledon and amnion, PGHS-1 expression does not increase with gestational age (44, 45). The ontogenic increase in PGHS-1 mRNA in cerebral vasculature is associated with a corresponding 3-fold increase in immunoreactive PGHS-1 in brain microvessels of the juvenile animal.

In contrast to PGHS-1, PGHS-2 mRNA was not readily detectable in various regions of the porcine brain. However, the highest PGHS-2 expression was observed in brain cortex and microvasculature of newborn animals. PGHS-2 mRNA levels decreased with age, and although immunoreactive PGHS-2 did not exhibit similar ontogenic changes, PGHS-1 protein and mRNA were markedly less in the newborn, thus disclosing the relative abundance of PGHS-2 in newborn brain and microvessels.

Further support for the suggestion that increased PGHS-2 expression accounts for the high prostaglandin G/H synthase activity and, in turn, high prostaglandin levels in newborn comes from studies using PGHS inhibitors. NS-398 is more than 100-fold more potent in inhibiting PGHS-2 than PGHS-1.
Furthermore, inhibition of PGHS-1 by NS-398 could be reversed by excess substrate, arachidonic acid, whereas time-dependent inactivation of PGHS-2 by NS-398 renders PGHS-2 refractory to substrate-mediated relief of inhibition (47). In this context, pretreatment of microvessels with NS-398 may have enabled us to differentiate PGHS-1 and PGHS-2 in their contribution toward prostanoid synthesis by cerebral microvasculature.

NS-398 decreased prostaglandin levels and synthesis by 35% in juvenile tissues and by 60% in those of the newborn. The differential effects of NS-398 in newborn animals compared with the juvenile pigs were consistently observed in prostaglandin synthesis by isolated cerebral vasculature, in prostaglandin levels in brain cortex, and in vivo cerebrovascular prostaglandin production. Moreover, unlike the brain prostaglandins, NS-398 minimally reduced prostanoids in the newborn retina (Fig. 5) in which PGHS-2 mRNA could not be detected (Fig. 1). Thus, the preponderance of PGHS-2 mRNA and protein in newborn cortex and microvasculature, minimal expression of immunoreactive PGHS-1 in the newborn, and pronounced inhibition of prostanoid synthesis both in vivo and in vitro by NS-398 in newborn tissues compared with those of juvenile animals, taken together, indicate that PGHS-2 is a major contributor to prostaglandin synthesis in newborn brain and cerebral microvasculature.

The factors responsible for the increased expression of PGHS-2 in newborn cerebral cortex and microvasculature are not known but may include estrogens which increase late in gestation and induce a gradual increase in PGHS activity (48, 49). Indeed, high PGHS-2 expression and concomitant increase in prostanoid synthesis in late gestation and at term was observed in fetal placental tissues (45). Besides being transcriptionally regulated by various stimuli, PGHS-2 expression is also controlled by factors affecting mRNA stability (50) and its translatability (51). The mechanisms governing the increased expression of PGHS-2 in neonatal brain remain to be elucidated.

The function of the elevated prostaglandin concentrations in the brain is so far not known. Because prostaglandins have been shown to exhibit neuroprotective properties (52), it is likely that their increased levels during the perinatal period may provide protection to the fetal brain toward the end of parturition when oxygen tension is markedly reduced (53) and the risk of hypoxic brain injury increases. Being a rapidly
inducible enzyme, PGHS-2 would be suited for such a temporary but important role in perinatal life.

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