Prostaglandin Biosynthesis and Catabolism in the Developing Fetal Sheep Brain*

(Received for publication, November 19, 1975)

Cecil Robert Pace-Asciak and G. Rangaraj

From the Research Institute, the Hospital for Sick Children, Toronto, Canada

The biosynthesis of prostaglandins E₂ and F₂₀, as well as the catabolism of [9β-¹³C]prostaglandin F₂₀, by homogenates of whole brain from fetal and neonatal lambs was investigated. The biosynthetic measurements utilized the mass spectrometric deuterium isotope dilution technique (Samuelsson, B., Hamberg, M., and Sweeteey, C. C. (1970) Anal. Biochem. 38, 301-304; Wolfe, L. S., and Pace-Asciak, C. (1972) in Prostaglandins in Fertility Control (Bergström, S., Green, K., and Samuelsson, B., eds) Vol 2, pp. 201-207, WHO). Our previous studies with the rat lung (1) and rat kidney (2) have shown distinct changes in the activity profiles of several enzymes of the prostaglandin catabolizing system as a function of animal maturity. Thus, the first two steps in the catabolizing system (PG 15-hydroxy dehydrogenase and PG 13-reductase) were more active in the immature animal as compared to the adult, although we have since discovered that the extent of this effect is quite organ- and species-dependent. Furthermore, a clear-cut dissociation of enzyme activity was observed in the rat kidney in which PG 15-hydroxy dehydrogenase and PG 13-reductase were more active than in the adult during the first 4 postnatal weeks (peak activity 60-fold around Day 19) dropping to adult levels by Day 40. Another enzyme, PG 9-hydroxy dehydrogenase, was absent in the fetal and neonatal kidney, appearing first around Day 20, and reaching adult levels by Day 49 (2). On the other hand, no significant change in biosynthesis took place between Day 10 or Day 20 and the adult (3). Based on the known vasoconstrictor properties of both E and F prostaglandins in the rat kidney, the cortical localization of the catabolizing system as well as the reported cortical blood flow redistribution in the rat kidney during the first 3 to 4 postnatal weeks, we interpreted the changing (increasing) prostaglandin catabolism as an important protective mechanism during corticogenesis, probably required to act against the potentially adverse effects of the prostaglandins formed locally (2).

Stimulated by recent reports implying a role for prostaglandins in cell differentiation (4, 5), we undertook the present investigation to determine whether prostaglandin biosynthesis and/or catabolism could be demonstrated in fetal brain.
and (b) whether these activities changed during brain development in a way that could be interpreted on the basis of the changing morphological events in this organ.

EXPERIMENTAL PROCEDURE

Materials

Unlabeled and deuterium-labeled prostaglandin standards were kindly supplied by Drs. J. E. Pike and U. Axen. The Upjohn Co. K-35-tritium-labeled 15-keto-H$_2$-PGF$_{\alpha}$, and PGF$_{\alpha}$, (specific activity = 90 Ci/μmol) were purchased from New England Nuclear, Boston. 5,6-Tritium-labeled 15-keto-H$_2$-PGF$_{\alpha}$ was prepared as reported previously (2). All solvents were of top quality reagent grade and distilled in glass before use (2).

Animals—Pregnant Suffolk ewes were anesthetized with methoxyflurane/nitrous oxide and the fetuses were delivered by cesarean section at the indicated days of gestation. Brains were rapidly dissected, cleaned from blood and meningeal tissue, rinsed with ice-cold 0.05 M KH$_2$PO$_4$/NaOH (pH 7.4), and used immediately for the assay of prostaglandin catabolism assay were similarly treated except that after localization of the radioactivity; another aliquot (1/) was assayed for distribution of radioactivity; another aliquot (1/) was assayed for distribution of radioactivity. That the endogenous compounds being measured represent PGE$_2$ and PGF$_{\alpha}$, and not an artifact was confirmed by computer-operated recording of mass spectra on each sample. Making use of the selected ion detection program in the computer, full spectra were recorded continuously during elution of the compounds from the gas chromatograph, and subsequently, several characteristic fragment ions specific to the deuterated and protonated prostaglandins were displayed as a function of spectrum number (representing retention time). A direct superimposition of the major fragment peaks in the sample with those in the deuterated compound was observed, confirming identity of the compounds.

The levels of PGE$_2$ and PGF$_{\alpha}$ in unincubated homogenates of brain were very low, approximately 65 pg/g of PGE$_2$ (two experiments) and 60 pg/g of PGF$_{\alpha}$, and not an artifact was confirmed by computer-operated recording of mass spectra on each sample. Making use of the selected ion detection program in the computer, full spectra were recorded continuously during elution of the compounds from the gas chromatograph, and subsequently, several characteristic fragment ions specific to the deuterated and protonated prostaglandins were displayed as a function of spectrum number (representing retention time). A direct superimposition of the major fragment peaks in the sample with those in the deuterated compound was observed, confirming identity of the compounds.

The levels of PGE$_2$ and PGF$_{\alpha}$, in unincubated homogenates of brain were very low, approximately 65 pg/g of PGE$_2$ (two experiments) and 60 pg/g of PGF$_{\alpha}$, (two experiments). As shown in Fig. 2, these levels rose about 4-fold in PGE$_2$ and about 3-fold in PGF$_{\alpha}$, during a 10-min incubation (37°), probably due to the presence of endogenous free arachidonic acid in the homogenate. A slight further increase results after addition of exogenous arachidonic acid (100 μg/g of wet tissue). All subsequent experiments, therefore, were carried out in the presence of excess exogenous arachidonic acid.

At all ages tested, even at gestational age 31 days, both PGE$_2$ and PGF$_{\alpha}$, were detected (Fig. 3). In all experiments, except at
The Prostaglandin System in Developing Lamb Brain

Catabolism of Prostaglandin F_2α—The 40-day fetal lamb brain metabolized PGF_2α quite actively with over 40% of tracer being metabolized into the 15-keto and 15-keto-H_2 metabolites in 10 min at 30° when NAD⁺ (4 mM) was present in the incubation mixture. Although both metabolites were formed, 15-keto-PGF_2α was the major compound, indicating that PG 15-hydroxy dehydrogenase was by far more active than PG 13-reductase. The conversion of PGF_2α into these metabolites was very responsive to substrate dilution, indicative of a low capacity saturable enzyme system (Fig. 4).

Fig. 5 shows the progression with age of the activity of the major prostaglandin-catabolizing enzyme (PG 15-hydroxy dehydrogenase) in the lamb brain. Unlike the biosynthetic measurements large differences in the prostaglandin-catabolizing capacity were observed with age. Clearly, highest activity is associated with the earliest fetus tested. A definite pattern is observed showing an inverse relationship of PG 15-hydroxy dehydrogenase activity with advancing age. Whereas the 32-day fetal brain possessed an active PG 15-hydroxy dehydrogenase with detectable PG 13-reductase, the newborn (Day 2) brain was almost completely devoid of catabolizing activity. The difference in activity between the 32-day fetus and term was approximately 12-fold.

Characterization of PG Metabolites—Several samples from the above experiments were combined and the 15-keto and 15-keto-H_2,15-keto-H_2-PGF_2α (15Kd-PGF_2α) (1 µg each) was added followed by a mixture of [5,6-3H]PGF_2α, PGF_2α, and 15-keto-H_2-PGF_2α (500,000 dpm each). Samples were purified as described in the text and assayed as shown in Fig. 1.

DISCUSSION

This paper describes for the first time the age profile of the prostaglandin biosynthetic and catabolizing enzymes in whole brain from fetal and neonatal lambs; the activity of these enzymes was monitored at several stages of brain development. That portion of the catabolizing system requiring NAD as co-

\[ \text{PGF}_2\alpha - \text{Me-TMSi} \]

\[ \text{PGE}_2 - \text{Me-MO-TMSi} \]

Fig. 1. Measurement of PGE_2 and PGF_2α in fetal lamb brain (Day 116) by mass fragmentography. Purified samples obtained from the experimental workup as methyl esters containing tetradeuterated carrier compounds and endogenous protonated compounds were converted into the corresponding O-methyl oxime (Me,Si), derivatives (PGE_2), (Me,Si)_2 derivatives (PGF_2α), or (Me,Si)_3 derivatives (15-keto-H_2-PGF_2α) as described under "Experimental Procedure." Measurements were made on a Varian MAT CH-5 gas chromatograph-mass spectrometer system equipped with a peak-matching unit capable of monitoring two fragment ions consecutively. Samples of PGF_2α-Me-O-methyl oxime (Me,Si)_2 were monitored at m/e 512 (d, carrier compound) and m/e 508 (d, endogenous compound); PGF_2α-Me,(Me,Si)_2 was monitored at m/e 527 (d, carrier compound) and m/e 508 (d, endogenous compound); PGF_2α-Me,(Me,Si)_3 derivatives (PGF_2α), or (Me,Si)_3 derivatives (15-keto-H_2-PGF_2α) were monitored at m/e 423 (d, carrier compound) and m/e 427 (d, endogenous compound); PGF_2α-Me,(Me,Si)_3 derivatives (PGF_2α), or (Me,Si)_3 derivatives (15-keto-H_2-PGF_2α) were monitored at m/e 423 (d, endogenous compound) and m/e 427 (d, endogenous compound); PGF_2α-Me(O-Me)_3 was monitored at m/e 427 (d, carrier compound) and m/e 423 (d, carrier compound); PGF_2α-Me(O-Me)_2 was monito
other brain proteins. The difference in protein measurements of PG 15-hydroxy dehydrogenase activity with advancing age of fetal fetuses and decreased with advancing age until it was almost undetectable at term (Figs. 4 and 5). The inverse relationship and because of active prostaglandin catabolism in peripheral organs (especially the lung and kidney). Although prostaglandins of maternal origin would not be expected to cross over into the fetal circulation due to active prostaglandin catabolism by the placenta (8), prostaglandins of fetal origin present in the fetal circulation could potentially be harmful to fetal organs unless active mechanisms were present to eliminate them. This is especially so during certain crucial stages of development (see below). Indeed, we have recently shown that some fetal lamb tissues (lung, liver, and kidney) have the capacity to form prostaglandins; yet these tissues, which are responsible for the major catabolism of circulating prostaglandins in the adult, do not possess much catabolizing activity at a gestational age of 32 to 40 days (3). Thus, it is quite probable that considerable amounts of prostaglandins of fetal origin are present in the fetal circulation, unlike in the adult. Since there is no blood-brain barrier at this age to exclude crossover of circulating prostaglandins into brain, some other form of rapid...

![Figure 4. Effect of age and substrate concentration on the catabolism of [3H]-PGF sub by homogenates of the sheep brain.](image)

![Figure 5. Activity profile of PG 15-hydroxy dehydrogenase (15-PGDH) in lamb brain as a function of age.](image)

Since the main changes with respect to the “prostaglandin system” that occur during brain development relate to changes in the activity of the catabolism rather than the biosynthesis of these compounds, we have termed this period of maximal change the “critical prostaglandin period.” This period appears to be different from one tissue to the next and from one species to the next. Thus the critical prostaglandin period in the rat kidney occurs postnatally around 19 days of age (2) whereas in the sheep kidney this period occurs prenatally (3). In the sheep brain the period of maximal change in prostaglandin catabolism appears around 30 days gestational age, whereas this was undetectable in the rat brain because PG 15-hydroxy dehydrogenase activity was exceedingly low.

When the present results are considered in relation to the development of the blood-brain barrier, it is interesting to note that an inverse relationship exists between the two. For example, highest PG catabolism in fetal lamb brain is evident at an age when the blood-brain barrier is virtually nonexistent, i.e. 31 days gestational age. As the fetus matures, a blood-brain barrier develops (7) and the activity of PG 15-hydroxy dehydrogenase decreases (Fig. 5). These results appear to suggest that high PG 15-hydroxy dehydrogenase activity in the young fetal brain is needed to protect itself from circulating prostaglandins to which the mature brain would not normally be exposed, both because of a well developed blood-brain barrier and because of active prostaglandin catabolism in peripheral organs (especially the lung and kidney). Although prostaglandins of maternal origin would not be expected to cross over into the fetal circulation due to active prostaglandin catabolism by the placenta (8), prostaglandins of fetal origin present in the fetal circulation could potentially be harmful to fetal organs unless active mechanisms were present to eliminate them. This is especially so during certain crucial stages of development (see below). Indeed, we have recently shown that some fetal lamb tissues (lung, liver, and kidney) have the capacity to form prostaglandins; yet these tissues, which are responsible for the major catabolism of circulating prostaglandins in the adult, do not possess much catabolizing activity at a gestational age of 32 to 40 days (3). Thus, it is quite probable that considerable amounts of prostaglandins of fetal origin are present in the fetal circulation, unlike in the adult.
protection must exist—PG 15-hydroxy dehydrogenase activity (Fig. 5). Furthermore, because mechanisms to extrude locally formed prostaglandins are probably not very efficient in the 30-day fetal brain, PG 15-hydroxy dehydrogenase activity might represent the type of rapidly needed inactivation to prevent any long lasting effects of these potent vasoconstrictor compounds (9) until other mechanisms for their extrusion develop. These mechanisms must become fully developed in the term fetus, because brain PG 15-hydroxy dehydrogenase is virtually nonexistent.

Prostaglandins have been shown to induce morphological differentiation of cells grown in culture (4, 5), although this effect appears to be restricted to the E-type compounds. In our experiments, PGE, is formed in amounts several-fold greater than PGF, Thus, mechanisms must be available for the rapid inactivation of the locally formed PGs as a protection against the premature induction of cell differentiation. In the lamb brain, a critical outburst of cell differentiation has been reported around a narrow period between 55 and 65 days gestation (10). The high levels of PG 15-hydroxy dehydrogenase observed (Fig. 5) in our study before this period thus appear to reveal an important mechanism for inactivation of prostaglandins, possibly serving as a mechanism for protection of the developing brain against the premature induction of cell differentiation by these compounds. Interestingly, subsequent to 75 days, PG 15-hydroxy dehydrogenase activity drops markedly to almost undetectable levels in the term fetus. It might be important to observe that although prostaglandins are still formed by the neonatal, and presumably also the adult, brain, PG 15-hydroxy dehydrogenase activity is virtually undetected, suggesting that alternate routes of inactivation and/or elimination take place in the more mature brain. These alternate routes of elimination are probably also present in the immature rat brain because PG 15-hydroxy dehydrogenase activity in this species is almost undetectable in brains from fetuses (gestational age 10 and 15 days), neonates (Days 1 and 13), and adult animals. Thus, brain PG 15-hydroxy dehydrogenase activity appears to be both age- and species-specific.

Acknowledgments—The authors wish to thank Mr. L. Marai for his expert assistance in recording mass spectra and in carrying out the mass fragmentography on the Medical Research Council regional facility at the Best Institute, Toronto. All animal surgery was kindly performed by Dr. P. Olley and Mr. F. Hamilton.

Note Added in Proof—The catabolizing system in these experiments was stimulated only by NAD. Other cofactors (NADH, NADP, NADPH, 4 mM) did not stimulate catabolism of either PGF or PGE, showing the absence of PG-9-keto reductase and type II-NADP-PG 15-hydroxydehydrogenase in our preparation.

REFERENCES
Prostaglandin biosynthesis and catabolism in the developing fetal sheep brain.
C R Pace-Asciake and G Rangaraj


Access the most updated version of this article at http://www.jbc.org/content/251/11/3381

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/11/3381.full.html#ref-list-1