Prostaglandin Synthetase in the Human Neonatal Kidney

ZVI FRIEDMAN, M.D., F.R.C.P.(C) AND LAURENCE M. DEMERS, Ph.D.

*Departments of Pediatrics and Pathology, The Milton S. Hershey Medical Center, The Pennsylvania State University
College of Medicine, Hershey, Pennsylvania, USA*

Summary

Prostaglandins play an important role in renal physiology. We have studied the prostaglandin synthetase system in 10 human newborn kidneys ranging from 22 to 40 weeks of gestation who succumbed within 40 hr postnatally. Slices of medulla and cortex were incubated within 2 hr of death in a medium containing 50 μ g arachidonic acid and 50 μ Ci [3 H]arachidonic acid. The incorporation of [3 H]AA into prostaglandin (PGE) and PGF was determined at 30-min intervals in medulla and cortex tissue and in the incubation media over 120 min and at 180 min, using silicic acid chromatography and scintillation counting. The study showed that biosynthesis of the primary prostaglandins occurs mainly in the renal medulla as early as 22 weeks of gestation, with the biosynthetic activity increasing concomitantly with advanced gestation. The ratio of PGE to PGF in the medulla and cortex was always greater than 1. The highest ratio was obtained in kidneys from neonates born before 28 weeks of gestation. Because prostaglandins are biosynthesized in the neonatal kidney, it is conceivable that hemodynamic and fluid and electrolyte homeostasis functions of prostaglandins may exist at this very early stage of development.

Speculation

Because prostaglandins are biosynthesized early in the development of the neonatal kidney, it is conceivable that hemodynamic and fluid and electrolyte homeostasis functions for prostaglandins might exist at this very early stage of development. Further information into the significance of these findings awaits measurement of age-dependent biologic activity profiles of prostaglandins and their metabolites and prostaglandin-metabolizing enzymes which influence the net production of renal prostaglandins.

The polyunsaturated fatty acid, arachidonic acid (AA), is the only defined substrate for prostaglandin synthesis in the human kidney (32, 40). In tissues, AA is found in ester linkage at the position 2 of glycerol in phospholipids and in cholesteryl esters and triglycerides. In order for AA to become available for enzymatic conversion to prostaglandins by prostaglandin synthetase, it must be released by the appropriate acyl hydrolases (6, 17, 19, 43). This reaction is believed to be the rate-limiting step in prostaglandin production *in vivo* (17, 19, 43). Intrarenal prostaglandin synthesis involves the conversion of AA via a cyclic-endoperoxide intermediate to prostaglandins E and F (12). The role of prostaglandin A in kidney remains to be elucidated because it may be formed as a dehydration product of PGE (27). Prostaglandin synthetase is a complex of enzymes which catalyzes the formation of prostaglandins in different cell types in the kidney (1, 14, 30). It was originally believed that renal prostaglandin synthesis occurred exclusively in the renal medulla and papilla (1, 5); however, recent experiments show that smaller amounts of prostaglandins are also produced in the renal cortex (18, 21, 38,

42). The presence of prostaglandins in small amounts in the renal cortex may be related to their limited synthesis and/or to their active metabolism and degradation in this area (2, 22).

Renal prostaglandins have been implicated in several physiological and pathophysiological processes including the regulation of blood pressure (24, 25), renal blood flow (27), salt and water homeostasis (46), and modulation of the renin-angiotensin-aldosterone axis (7, 11, 23, 28, 39).

Although biosynthesis of prostaglandins by the fetal kidney in various species has been reported (34, 44), it has been inadequately investigated in the human kidney during the early stages of development. We therefore determined the activity of the prostaglandin synthetase system in the human neonatal kidney at different ages of gestation.

MATERIALS AND METHODS (47)

PATIENTS

Clinical data for the study groups are shown in Table 1. Prostaglandin biosynthesis was studied in 10 neonatal kidneys. Their gestational age ranged from 22 to 40 weeks as estimated according to the last menstrual period and physical examination (10). Patients 1 and 2 were born following spontaneous abortion. Birth weights ranged from 400 to 3450 g. All infants were cared for in the Neonatal Intensive Care Unit at The Milton S. Hershey Medical Center and died within 2 days postnatally. There was no obvious clinical or histologic evidence of abnormal renal function. Neither mothers nor infants received any drug known to inhibit prostaglandin production.

METHODS

Kidneys were removed from the neonates within 60 min following death. The medulla and cortex were carefully dissected, sliced, weighed, and prepared for *in vitro* incubations. Slices were incubated in flasks containing 1 ml of medium 199 (48) per 10 mg tissue. One hundred μ g of indomethacin (49) (dissolved in dimethyl sulfoxide) were added to incubated control tissues. The flasks were gassed with 95% O₂:5% CO₂ and shaken at 60 rpm in a Dubnoff metabolic incubator at 37°C. Fifty ml of the incubation medium contained 50 μ g of arachidonic acid and 50 μ Ci of [3 H]-labeled arachidonic acid (New England Nuclear, Boston, MA) as previously described (4, 13). The incorporation of [3 H]arachidonic acid into PGE, PGF, and PGA was determined at 30-min intervals in the medulla and cortical tissues and in the incubation medium up to 120 min and at 180 min. The enzymatic reaction was stopped by the immersion of the flask into an ice bath. The medium and tissue were separated by centrifugation at 4°C at 250 g. Five ml of the medium were mixed and vigorously shaken with 3 ml of ethyl acetate:isopropanol:0.1 N HCl, 3:3:1. The mixture was centrifuged, and the upper ethyl acetate layer was transferred to conical centrifuge tubes and stored under nitrogen at -20°C until

Table 1. Clinical data for study groups

Patients	Sex		Gestational Age (wk)	Birth Weight (g)	Postnatal Age at Time of Study (hr)	Diagnosis
	M	F				
1. M	1		22	400	2.5	Atelectasis, immaturity
2. M	1		22	435	2.0	Atelectasis, immaturity
3. L		1	26	820	10.0	HMD ¹
4. Z	1		26	845	26.0	HMD, ICH
5. K		1	26	780	18.0	HMD
6. M	1		28	1040	32.0	HMD, pneumopericardium
7. B	1		28	1120	22.0	HMD, ICH
8. T	1		32	1660	40.0	HMD, ICH
9. H	1		40	3450	16.0	Interrupted aortic arch
10. B		1	40	3380	36.0	Asphyxia, meconium aspiration
Range			22-40	400-3450	2.0-40	
Mean $\pm$ SEM			29 $\pm$ 2.0	1393 $\pm$ 355	20.5 $\pm$ 6.5	

¹ HMD, hyaline membrane disease; ICH, intracranial hemorrhage.

analysis. Tissues were homogenized with phosphate-buffered saline, pH 7.4 (0.05 M $\text{KH}_2\text{PO}_4/\text{NaOH}$, pH 7.4), and the extraction procedure was identical to that of the medium. Isolation of the PGE, PGF, and PGA and free [^3H]arachidonic acid was carried out using silicic acid chromatography to separate the individual prostaglandins followed by liquid scintillation (9). Because of the possibility that a proportion of the exogenous AA may be converted to prostaglandin-like compounds during the incubation and subsequent extraction and separation procedures in the absence of active enzyme in the incubation medium (3, 31), the medium with AA was analyzed for prostaglandins and served as a blank. The final results are reported after subtraction of the blank from the counts obtained from the incubation of medulla and cortex tissues and medium.

RESULTS

Figure 1 illustrates the biosynthesis of prostaglandins E and F in the medium of incubated renal medulla. Because of the suggestion that prostaglandin A may be an artifact resulting from dehydration of prostaglandin E during the extraction procedure (27), the counts for prostaglandins E and A are combined and represented as prostaglandin E. It is apparent that the primary prostaglandin E is synthesized by incubated medulla slices of kidneys obtained from neonates delivered as early as 22 weeks of gestation and that the primary prostaglandin F is synthesized as early as 26 weeks of gestation. The biosynthetic activity of the prostaglandins E and F increases proportionally with advancing gestation. The biosynthesis of prostaglandin E is always higher than that of prostaglandin F, and the ratio of the individual prostaglandins E to F is higher in the renal medulla medium obtained from infants born before 28 weeks of gestation. Maximal biosynthetic activity was obtained during incubation periods of 60 and 90 min. Figure 2 illustrates the biosynthesis of prostaglandins E and F in the medium of renal cortex. Although a similar pattern of prostaglandins E and F biosynthesis is found in the incubation medium of renal medulla and cortex, the levels obtained from the renal cortex are less than 50% of that of the renal medulla. The decreased values of PGE and PGF that were obtained at 120 and 180 min of renal medulla and cortex incubation could represent an increase in the production of PG metabolites. Biosynthesis of the primary prostaglandins E and F from the cortical and medullary tissues and from all the incubations containing indomethacin was less than 5% of the primary prostaglandins measured in the incubation medium of these tissues.

DISCUSSION

This study shows that *in vitro* biosynthesis of the primary prostaglandin E in the renal medulla and cortex occurs as early as 22 weeks of gestation and that the primary prostaglandin F is

synthesized as early as 26 weeks of gestation. The term "prostaglandin synthetase" has been used to refer to a system that converts certain free polyunsaturated fatty acids, namely arachidonate in our study, to stable prostaglandin products, mainly PGE and PGF. The recovery of prostacyclin and its stable metabolite 6-keto-PGF $_{1\alpha}$ from fetal tissues including kidney was previously documented (36). It is conceivable that in the method used in our study, prostacyclin and/or 6-keto-PGF $_{1\alpha}$ were eluted together with PGE (15). The synthetase system is a complex of enzymes which include a fatty acid cyclo-oxygenase step and subsequent isomerization and reduction steps. In an *in vitro* system like the one described above, the latter steps are rate limiting. *In vivo*, however, the availability of the substrate fatty acid is the rate-limiting step, and the release of arachidonic acid by phospholipase A_2 and other acyl hydrolases is the regulator of PG production (6, 17, 19, 43). Further differences between *in vitro* and *in vivo* studies reside in the fact that although our patients did not show any clinical or histologic evidence of renal involvement, periods of hypotension during life as well as during the time between the pronouncement of death and extraction of the kidney which may be designated as "warm ischemia time" leading to renal ischemia and ischemic kidney appear to release increased levels of prostaglandins (29). The extraction of PGA and PGF from a whole kidney preparation of 10- and 16-week-old human fetuses (8) and the production of prostaglandins from fetal lambs and rat kidneys were previously reported (34, 44).

This study demonstrates that the renal medulla is the main site for the synthesis of the primary PGE and PGF and that lower levels of prostaglandins were extracted from renal cortical tissues. Similar results were reported in human adult kidney by Spector *et al.* (38), who found a steep gradient for PGE, PGF, and PGA with the papilla containing the heaviest concentration and the cortex the least, and in animals (1, 5, 18, 21, 38, 42). Jovenaz *et al.* (16) found that the actual prostaglandin content of fresh tissue is low and merely reflects the capacity of the tissue to biosynthesize prostaglandins during the period between killing the organism and actually extracting the tissue. The absence of renal storage of prostaglandins bears upon the various reports of tissue content of these compounds (31, 37). If the appropriate fatty acid substrate is available for the synthesis of prostaglandins such as in our study, then depending on the methods used to obtain and extract the tissue, the reported content may be more a reflection of potential biosynthetic activity than of the absolute content. The common denominator in these stimuli which range from physiologic to the pathophysiologic appears to be related to some element of cellular damage or dysfunction. It has also been suggested that phospholipase A_2 , which may have a role in initiating prostaglandin synthesis, may participate in the alterations in cell membranes that occur as a result of many of the stimuli known to lead to prostaglandin release. The presence of prostaglandins in small amounts in the renal cortex compared with the renal medulla may

Fig. 1. Biosynthesis of prostaglandins E and F in renal medulla medium (mean $\pm$ S.D.). Samples were obtained from neonates born at 22 to 40 weeks of gestation (groups 1 to 5) and who succumb within 40 hr postnatally. The incorporation of [³H]arachidonic acid into the prostaglandins E and F was measured during the incubation periods of 30 to 180 min.

Fig. 2. Biosynthesis of prostaglandins E and F in renal cortex medium (mean $\pm$ S.D.). Legend as in Figure 1.

be related to their limited synthesis and/or to their active metabolism and degradation in this area (2, 22, 24). Pace-Asciak (33, 34) reported a rapid postnatal rise in prostaglandin catabolism with no change in the overall biosynthesis of prostaglandins by

the rat kidney. The transit of prostaglandin from sites of medullary synthesis to the urine to sites of cortical inactivation or into the venous and/or lymphatic circulation has not been thoroughly defined.

The decrease in prostaglandin synthesis after 60 to 90 min of incubation may not result solely from substrate depletion. Prostaglandin synthetase activity has been reported to decline rapidly in both broken cells (35) and in crude enzyme preparations (45). Self-catalyzed inactivation of prostaglandin synthetase has also been suggested (20). The inhibition of prostaglandin biosynthesis after the addition of indomethacin is not surprising because this drug is known to inhibit prostaglandin cyclooxygenase activity (41).

In our study, the ratio of prostaglandin E to prostaglandin F in the medium of renal medulla and cortex is always greater than 1; the highest ratio was obtained in kidneys from neonates born before 28 weeks of gestation. Similar ratios of PGE and PGF were reported in fetal lambs (44).

The finding of prostaglandin synthesis early in the development of the neonatal kidney is of interest because PGE and PGF are believed to be important mediators of blood pressure (24, 25), the rate and distribution of renal blood flow (27), salt and water homeostasis (46), as modulators of the renin-angiotensin-aldosterone axis (7, 11, 23, 28, 39), and as potentiators of adrenergic nerve activity (26, 46). Inasmuch as prostaglandins are biosynthesized in the neonatal kidney, it is conceivable that such hemodynamic and fluid and electrolyte homeostatic functions for prostaglandins might exist at this very early stage of development. Further information into the significance of these findings awaits measurement of age-dependent biological activity profiles of prostaglandins and their metabolites and prostaglandin-metabolizing enzymes which influence the net production of renal prostaglandins.

REFERENCES AND NOTES

1. Ånggård, E., Bohman, S. O., Griffin, J. E. III, Larsson, C., and Maunsbach, A. B.: Subcellular localization of the prostaglandin system in the rabbit renal papilla. *Acta Physiol. Scand.*, **84**: 231 (1972).
2. Ånggård, E.: Studies on the analysis and metabolism of the prostaglandins. *Ann. N. Y. Acad. Sci.*, **180**: 200 (1971).
3. Ånggård, E., and Jonsson, C. E.: Efflux of prostaglandins from scalded tissue. *Acta Physiol. Scand.*, **81**: 440 (1971).
4. Bohman, S. O., and Larsson, C.: Prostaglandin synthesis in membrane fractions from the rabbit renal medulla. *Acta Physiol. Scand.*, **94**: 244 (1975).
5. Crowshaw, K.: The incorporation of [14 C]arachidonic acid into the lipids of rabbit renal slices and conversion to prostaglandins E_2 and $F_{2\alpha}$. *Prostaglandins*, **3**: 607 (1973).
6. Danon, A., Heimberg, M., and Oates, J. A.: Enrichment of rat tissue lipids with fatty acids that are prostaglandin precursors. *Biochim. Biophys. Acta*, **388**: 318 (1975).
7. Danon, A., Chang, L. C. T., Sweetman, B. J., Nies, A. S., and Oates, J. A.: Synthesis of prostaglandins by the rat renal papilla *in vitro* by the mechanism of stimulation by angiotensin II. *Biochim. Biophys. Acta*, **388**: 71 (1975).
8. Day, N. A., Attallah, A. A., and Lee, J. B.: Presence of prostaglandin A and F in fetal kidney. Letter to the Editor. *Prostaglandins*, **5**: 491 (1974).
9. Demers, L. M., and Gabbe, S. G.: Placental prostaglandin levels in preeclampsia. *Am. J. Obstet. Gynecol.*, **126**: 137 (1976).
10. Dubowitz, L., Dubowitz, V., and Goldberg, C.: Clinical assessment of gestational age in the newborn infant. *J. Pediatr.*, **77**: 1 (1970).
11. Friedman, Z., Demers, L. M., Marks, K. H., Uhrmann, S., and Maisels, M. J.: Urinary excretion of prostaglandin E following the administration of furosemide and indomethacin to sick low birthweight infants. *J. Pediatr.*, **93**: 512 (1978).
12. Hamberg, M., and Samuelsson, B.: Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.*, **70**: 899 (1973).
13. Hamberg, M.: Biosynthesis of prostaglandins in the renal medulla of rabbit. *FEBS Lett.*, **5**: 127 (1969).
14. Janszen, F. H. A., and Nugteren, D. H.: Histochemical localization of prostaglandin synthetase. *Histochemie*, **27**: 159 (1971).
15. Johnson, R. A., Morton, D. R., Kinner, J. H., Gorman, R. R., McGuire, J. C., Sun, F. F., Whittaker, N., Bunting, S., Salmon, J., Muncada, S., and Vane, J. R.: The chemical structure of prostaglandin x (prostaglyclin). *Prostaglandins*, **12**: 915 (1976).
16. Jovenaz, G. H., Nugteren, D. H., Beerthuis, R. K., and Van Dorp, D. A.: A sensitive method for the determination of prostaglandins by gas chromatography with electron-capture detection. *Biochim. Biophys. Acta*, **202**: 231 (1970).
17. Kunze, H., and Vogt, W.: Significance of phospholipase A for prostaglandin formation. *Ann. N. Y. Acad. Sci.*, **180**: 123 (1971).
18. Landolt, R., Shaw, S. M., Vetter, R. J., and Chen, S. H.: An autoradiographic study of prostaglandin E_1 and/or its metabolites in mouse kidney tissue. *Prostaglandins*, **5**: 305 (1974).
19. Lands, W. E. M., and Samuelsson, B.: Phospholipid precursors of prostaglandins. *Biochim. Biophys. Acta*, **164**: 426 (1968).
20. Lands, W. E. M., LeTellier, P. R., Rome, L. H., and Vanderhoek, J. Y.: Inhibition of prostaglandin biosynthesis. *Adv. Biosci.*, **9**: 15 (1973).
21. Larsson, C., and Ånggård, E.: Distribution of prostaglandin metabolizing enzymes in tissues of the swine. *Acta Pharmacol. Toxicol.*, **28** (Suppl. 1): 61 (1970).
22. Larsson, C., and Ånggård, E.: Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur. J. Pharmacol.*, **21**: 30 (1973).
23. Larsson, C., Weber, P., and Ånggård, E.: Arachidonic acid increases and indomethacin decreases plasma renin activity in the rabbit. *Eur. J. Pharmacol.*, **28**: 391 (1974).
24. Lee, J. B., Covino, B. G., Takman, B. H., and Smith, E. R.: Renomedullary vasodepressor substance, medullin. Isolation, chemical characterization and physiological properties. *Circ. Res.*, **17**: 57 (1965).
25. Lee, J. B.: The renal prostaglandins and blood pressure regulation. *Adv. Prostaglandins Thromboxane Res.*, **2**: 573 (1976).
26. McDonald, K. M., Miller, P. D., Anderson, R. J., Berl, T., and Schrier, R. W.: Hormonal control of renal water excretion. *Kidney Int.*, **10**: 38 (1976).
27. McGiff, J. C., Crowshaw, K., and Itskovitz, H. D.: Prostaglandins and the renal function. *Fed. Proc.*, **33**: 39 (1974).
28. McGiff, J. C., Crowshaw, K., Terragno, N. A., and Lonigro, A. J.: Renal prostaglandins: possible regulators of the renal actions of pressor hormones. *Nature (Lond.)*, **227**: 1255 (1970).
29. McGiff, J. C., Crowshaw, K., Terragno, N. A., Lonigro, A. J., Strand, J. C., Williamson, M. A., Lee, J. B., and Ng, K. K. F.: Prostaglandin-like substances appearing in canine renal venous blood during renal ischemia: their partial characterization by pharmacologic and chromatographic procedures. *Circ. Res.*, **27**: 765 (1970).
30. Muirhead, E. E., Germain, G., Leach, B. E., Pitcock, J. A., Stephenson, P., Brooks, B., Brosius, W. L., Daniels, E. G., and Hinman, J. W.: Production of renomedullary prostaglandins by renomedullary interstitial cells grown in tissue culture. *Circ. Res.*, **31** (Suppl. 2): 161 (1972).
31. Nugteren, D. H., Vonkeman, H., and Van Dorp, D. A.: Non-enzymatic conversion of all *cis*-8,11,14-eicosatrienoic acid into prostaglandin E_1 . *Recl. Trav. Chim. Pays-Bas. Belg.*, **86**: 1237 (1967).
32. Pace-Asciak, C., Cocci, F., and Wolfe, L. S.: Comparison of the conversion of arachidonic acid into prostaglandins by some tissues. *Proc. Can. Fed. Biol. Sci.*, **10**: 80 (1967).
33. Pace-Asciak, C. R.: Biosynthesis and Catabolism of Prostaglandins during Animal Development. *Adv. Prostaglandin Thromboxane Res.*, **1**: 35 (1976).
34. Pace-Asciak, C.: Activity profiles of prostaglandin 15 and 9 hydroxydehydrogenase and 13-reductase in the developing rat kidney. *J. Biol. Chem.*, **250**: 2795 (1975).
35. Pace-Asciak, C.: Prostaglandin synthetase activity in the rat stomach fundus. Activation by L-norepinephrine and related compounds. *Biochim. Biophys. Acta*, **280**: 161 (1972).
36. Pace-Asciak, C. R., and Rangaraj, G.: Distribution of prostaglandin biosynthetic pathways in organs and tissues of the fetal lamb. *Biochim. Biophys. Acta*, **528**: 512 (1978).
37. Piper, P. J., and Vane, J. R.: The release of prostaglandins from lung and other tissues. *Ann. N. Y. Acad. Sci.*, **180**: 363 (1971).
38. Spector, D., Zusman, R. M., Caldwell, B. V., and Speroff, L.: The distribution of prostaglandins A, E and F in the human kidney. *Prostaglandins*, **6**: 263 (1974).
39. Tan, S. Y., and Mulrow, P. J.: Inhibition of the renin-aldosterone response to furosemide by indomethacin. *J. Clin. Endocrinol. Metab.*, **45**: 174 (1977).
40. Van Dorp, D. A., Beerthuis, R. K., Nugteren, D. H., and Vonkeman, H.: The biosynthesis of prostaglandin. *Biochim. Biophys. Acta*, **90**: 204 (1964).
41. Vane, J. R.: Prostaglandins and aspirin-like drug. *Pharmacology and the Future of Man* (Proc. 5th Int. Congr. Pharmacol., San Francisco), Vol. 5, Basel, Karger, 1973, pages 352-378.
42. Vance, V. K., Attallah, A., Prezyna, A., and Lee, J. B.: Human renal prostaglandins. *Prostaglandins*, **3**: 647 (1973).
43. Vonkeman, H., and Van Dorp, D. A.: The action of prostaglandin synthetase on 2-arachidonylethanol. *Biochim. Biophys. Acta*, **164**: 430 (1968).
44. Walker, D. W., and Mitchell, M. D.: Prostaglandins in urine of foetal lambs. *Nature (Lond.)*, **271**: 161 (1978).
45. Wallach, D. P., and Daniels, E. G.: Properties of a novel preparation of prostaglandin synthetase from sheep seminal vesicles. *Biochim. Biophys. Acta*, **231**: 445 (1971).
46. Zins, G. R.: Renal prostaglandins. *Am. J. Med.*, **58**: 14 (1975).
47. Informed consent was obtained from the parents of each child and the experimental protocol was approved by the Clinical Investigation Committee at The Milton S. Hershhey Medical Center, The Pennsylvania State University College of Medicine.
48. Grand Island Biological Company, Grand Island, New York.
49. Merck, Sharp and Dohme, West Point, Pennsylvania.
50. This research was supported by NICHD Grant R01 HD11255-01.
51. Present and mailing address: Zvi Friedman, M.D., Department of Pediatrics, Baylor College of Medicine, Houston, Texas 77030.
52. Received for publication December 13, 1978.
53. Accepted for publication April 24, 1979.