Distribution of Prostaglandin E 9-Ketoreductase and Types I and II 15-Hydroxyprostaglandin Dehydrogenase in Swine Kidney Medulla and Cortex†

Sheng-Chung Lee,‡ Sheng-Shung Pong, David Katzen, Kung-Yue Wu, and Lawrence Levine*

ABSTRACT: Prostaglandin E 9-ketoreductase, NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (type I), and $NADP^+$-dependent 15-hydroxyprostaglandin dehydrogenase (type II) have been partially purified from swine renal medulla and cortex. Eleven times more NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity was found in the cortex than in the medulla. On the other hand, about twice as much $NADP^+$-dependent dehydrogenase activity was found in the medulla than in the cortex. The prostaglandin 9-ketoreductase activities were equally distributed in the swine kidney cortex and medulla.

The biological inactivation of prostaglandin is catalyzed by a NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (Anggard and Samuelsson, 1966). In the kidney, metabolism of prostaglandins by 15-hydroxyprostaglandin dehydrogenase occurs predominantly in the cortex with activities ten times those found in the medulla (Anggard and Larsson 1971; Larsson and Anggard, 1973). Prostaglandin synthetic activity, on the hand, is much higher in the medulla then in the cortex (Larsson and Anggard, 1973). Thus, there appears to be a regional distribution in the kidney for the formation and metabolism of the prostaglandins.

Recently, a second type of 15-hydroxyprostaglandin dehydrogenase, which is $NADP^+$ dependent, was found in several organs including kidney (Lee and Levine, 1974a,b). This $NADP^+$-dependent dehydrogenase, called type II dehydrogenase, was partially purified from monkey brain (Lee and Levine, 1974b). We now report the regional distribution of not only the two types of 15-hydroxyprostaglandin dehydrogenases, but also the enzyme that converts prostaglandin E to F, prostaglandin E 9-ketoreductase (Lee and Levine, 1974a), in the medulla and cortex of swine kidney.

Materials and Methods

Antiserum. Preparation and specificities of the monkey antibodies to 15-keto-$PGF_{2\alpha}$[1] and rabbit antibodies to $PGF_{2\alpha}$ have been described (Levine and Gutierrez-Cernosek, 1972 and Levine *et al.*, 1971).

Radioimmunoassays. Monkey antiserum (2 μl) was incubated with 15-keto-[^{3}H]$PGF_{2\alpha}$ in the presence or absence of inhibitor for 1 hr at 37°. Rabbit-antimonkey-γ-globulin, which served as the precipitating antigen-antibody system to separate bound from free prostaglandin, was then added to the reaction mixture and after incubation overnight at 2–4°, the precipitate containing the antibody-bound 15-keto-[^{3}H]$PGF_{2\alpha}$ was collected by centrifugation at 1,000*g* for 30 min. The precipitate was dissolved in 0.2 ml of 0.1 N NaOH and counted for radioactivity in a modified Bray's solution with a Packard liquid scintillation spectrometer. For analysis of $PGF_{2\alpha}$, diluted rabbit antiserum and goat-antirabbit-γ-globulin were used for radioimmunoassay by the double antibody procedure (Levine *et al.*, 1971).

Biologicals and Chemicals. [^{3}H]$PGF_{2\alpha}$ was purchased from New England Nuclear (Boston, Mass). Unlabeled prostaglandins and prostaglandin metabolites were a gift from Dr. Udo Axen of the Upjohn Company (Kalamazoo, Mich.). Pyridine nucleotides were purchased from Sigma Co. Swine kidneys, obtained from a slaughter house, were dissected into regions of medulla and cortex and stored at −70° until used. 15-Keto-[^{3}H]$PGF_{2\alpha}$ was prepared by treating [^{3}H]$PGF_{2\alpha}$ with partially purified dog lung 15-hydroxyprostaglandin dehydrogenase (Lee and Levine, 1974b).

Enzyme Assay. The standard assay mixture for determining 15-hydroxyprostaglandin dehydrogenase activity contained 1 mM $NADP^+$ or NAD^+, 1 μg of $PGF_{2\alpha}$, 0.1 M Tris-HCl buffer (pH 7.8), and enzyme in a final volume of 0.1 ml. For prostaglandin E 9-ketoreductase assay, the reaction contained 1 mM NADPH, 1 μg of PGE_2, 0.1 M Tris-HCl (pH 7.4), and enzyme in a final volume of 0.1 ml. The reaction mixtures were incubated at 37° for 10 min, and the enzymatic reaction was stopped by addition of 1 ml of cold Tris-HCl buffer (0.01 M, pH 7.4, containing 0.1% gelatin and 0.14 M NaCl) and incubation of this diluted solution in a boiling water bath for 2 min. After centrifugation to remove denatured protein, the clear supernatant fluid was analyzed for 15-keto-$PGF_{2\alpha}$ (15-hydroxyprostaglandin dehydrogenase activity) or $PGF_{2\alpha}$ (prostaglandin E 9-ketoreductase activity) by radioimmunoassay. The enzymatic activities are expressed as nanomoles of product generated per 10 min at 37°. Protein was determined by Lowry's method (Lowry *et al.*, 1951).

Enzyme Preparations. The two 15-hydroxyprostaglandin dehydrogenases and the prostaglandin reductase were par-

† Publication No. 986 from the Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received August 26, 1974.* This work was supported by Grant IM-22M from the American Cancer Society and by Grant HD-07966 from the National Institute of Child Health and Development. L.L. is an American Cancer Society Professor of Biochemistry (Award No. PRP-21).

‡ Present address: Department of Surgery, Division of Tumor and Transplant Immunology, Stanford University School of Medicine, Stanford, California 94305.

[1] Abbreviations used are: PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; 15-keto-$PGF_{2\alpha}$, 15-ketoprostaglandin $F_{2\alpha}$; PGE, prostaglandin E.

TABLE I: Partial Purification of Types I and II 15-Hydroxyprostaglandin Dehydrogenases and PGE 9-Ketoreductase from Swine Kidney Cortex.

Fraction	Total Protein (mg)	Total Activity (units)			Specific Activity (Unit/mg of Protein)			Yield (%)		
		Dehydrogenase		PGE 9-Keto-reductase	Dehydrogenase		PGE 9-Keto-reductase	Dehydrogenase		PGE 9-Keto-reductase
		Type I	Type II		Type I	Type II		Type I	Type II	
10,000*g* supernatant fluid	234.0	25.5	16.5	42.5	0.11	0.07	0.18	100	100	100
78,000*g* supernatant fluid	186.1	18.1	10.2	37.9	0.10	0.06	0.20	71	62	88
Ammonium sulfate (30–60%)	72.8	14.7	12.9	18.4	0.20	0.18	0.25	58	78	43
DEAE-Sephadex										
I	8.1	13.2			1.63			52		
II	11.7		10.9	16.8		0.93	1.44		66	40

TABLE II: Partial Purification of Type II 15-Hydroxyprostaglandin Dehydrogenase and PGE 9-Ketoreductase from Swine Kidney Medulla.

Fraction	Total Protein (mg)	Total Activity (units)		Specific Activity (unit/mg of Protein)		Yield (%)	
		Dehydro-genase	PGE 9-Keto-reductase	Dehydro-genase	PGE 9-Keto-reductase	Dehydro-genase	PGE 9-Keto-reductase
10,000*g* supernatant fluid	241.0	31.4	35.9	0.13	0.15	100	100
78,000*g* supernatant fluid	165.0	38.7	33.8	0.23	0.20	123	94
Ammonium sulfate (30–60%)	53.0	30.5	27.8	0.58	0.52	97	77
DEAE-Sephadex	10.2	40.8	19.8	4.00	1.94	130	55

tially purified from swine kidney medulla and cortex by ammonium sulfate fractionation of the particle free supernatant fluids and by DEAE-Sephadex chromatography. Swine kidney cortex and medulla (100 g) were homogenized in 0.092 M phosphate buffer (pH 7.3) containing 0.1 mM dithiothreitol with a Sorval Omni mixer that was operated at top speed for 2 min in ice-water; after stopping the mixer for 2 min, they were homogenized for an additional 2 min. The resulting suspensions were centrifuged at 10,000*g* for 20 min. The supernatant fluids were then centrifuged at 78,000*g* for 1 hr in a Spinco Model L2-65B ultracentrifuge with the use of a SW 50.1 rotor. Solid ammonium sulfate was added with stirring to the clear supernatant fluid to 30% saturation. After the mixture was stirred for 30 min at 0°, the precipitate was removed by centrifugation at 10,000*g* for 20 min. The supernatant fluid was then brought to 60% saturation of ammonium sulfate and equilibrated again for 30 min. The precipitate, insoluble in 60% ammonium sulfate, was collected by centrifugation (10,000*g* for 20 min) and was dissolved in phosphate buffer (0.092 M, pH 7.3, 0.1 mM dithiothreitol) and dialyzed against 100 volumes of this phosphate buffer. The dialysate was applied to a DEAE-Sephadex column that had previously been equilibrated with the phosphate buffer. The column was then washed with 1 column volume of phosphate buffer followed by elution with 4 column volumes of phosphate buffer containing a 0–1 M KCl linear gradient.

Results and Discussion

The yields and the increases in specific activities during the purification of the two 15-hydroxyprostaglandin dehydrogenases and the PGE 9-ketoreductase from swine renal medulla and cortex by ammonium sulfate fractionation and DEAE-Sephadex chromatography are shown in Tables I and II. As can be seen by the specific activities in the homogenates and cytoplasmic fractions, PGE 9-ketoreductase activity was distributed about equally between cortex and medulla. The $NADP^+$-dependent 15-hydroxyprostaglandin dehydrogenase (type II) activity was slightly higher in the medulla than in the cortex. Type II 15-hydroxyprostaglandin dehydrogenase activities were calculated assuming that $NADP^+$ dependence was absolute and the distribution was determined assuming that the cortex preparation was not contaminated with medulla or the medulla was not contaminated with the cortex.

The cortex has been shown to contain at least ten times more NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity than the medulla (Anggard and Larsson

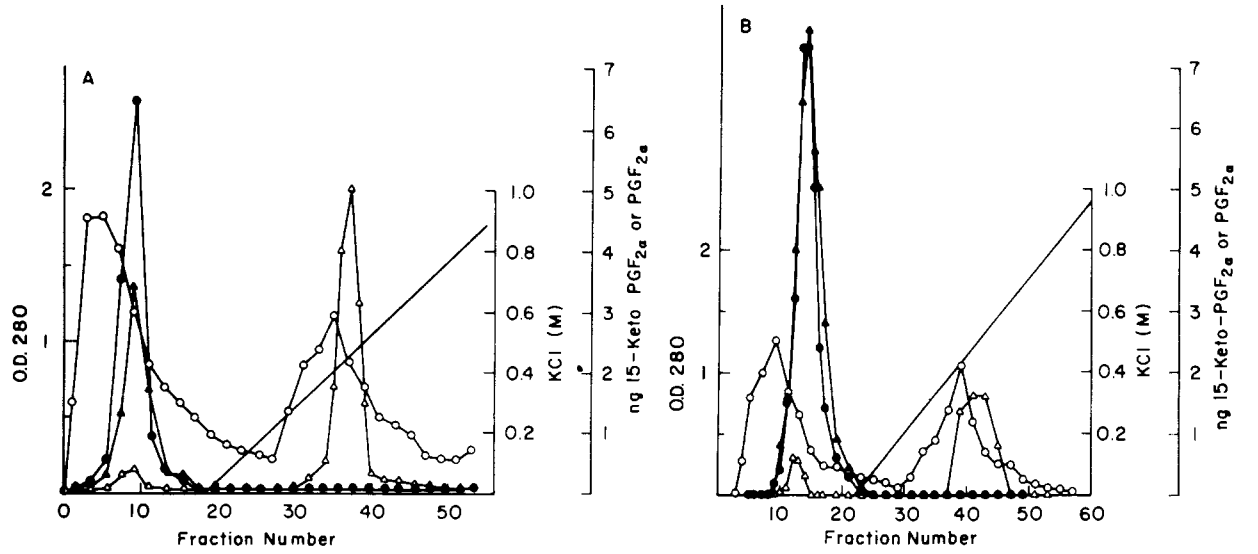

FIGURE 1: DEAE-Sephadex chromatography of swine kidney cortex (A) and medulla (B). (A) The 30–60% ammonium sulfate fraction (5.6 ml) from 5 g of swine kidney cortex was applied to a 1 × 12 cm DEAE-Sephadex column. The column was washed with 30 ml of 0.092 M phosphate buffer and eluted with 70 ml of the same buffer containing a linear gradient of KCl from 0 to 1 M. Fractions of 1.8 ml were collected for assay of 15-hydroxyprostaglandin dehydrogenase; 20-μl aliquots were used in a reaction volume of 0.1 ml containing 1 mM $NADP^+$ (▲) or NAD^+ (△) and 1 μg of $PGF_{2\alpha}$. The activity of prostaglandin E 9-ketoreductase was assayed with 20 μl of the fraction in a reaction volume of 0.1 ml containing 1 mM NADPH and 1 μg of PGE_2 (●). The reaction mixtures were incubated 20 min at 37° and the reaction was stopped by boiling for 2 min, followed by dilution with 0.5 ml of Tris-HCl buffer; 0.1 ml was assayed for 15-keto-$PGF_{2\alpha}$ and $PGF_{2\alpha}$. OD_{280} (○); KCl gradient (—). (B) The 30–60% ammonium sulfate fraction (8.5 ml) from 9 g of swine kidney medulla was applied to a 1.5 × 25 cm DEAE-Sephadex column. After washing with 50 ml of 0.092 M phosphate buffer, the enzyme was eluted with 160 ml of phosphate buffer (0.092 M) containing a 0–1 M KCl linear gradient. Fractions of 4.5 ml were collected, and 50 μl aliquots were assayed in a reaction volume of 0.1 ml containing 1 mM $NADP^+$ (▲) or NAD^+ (△) and 1 μg of $PGF_{2\alpha}$. For assay of prostaglandin E 9-ketoreductase, the 0.1-ml reaction mixtures contained 50 μl of enzyme, 1 mM NADPH, and 1 μg of PGE_2 (●). After incubation for 10 min at 37°, the reaction was stopped by boiling for 2 min, and was diluted with 0.5 ml of Tris-HCl buffer and 0.1 ml was assayed for 15-keto-$PGF_{2\alpha}$ or $PGF_{2\alpha}$. OD_{280} (○); gradient (—).

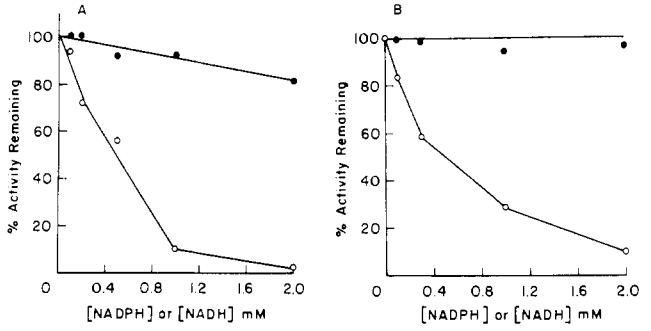

FIGURE 2: Inhibition of type I and type II 15-hydroxyprostaglandin dehydrogenases by NADPH and NADH. (A) Type I enzyme (16 μg), after DEAE-Sephadex chromatography, was incubated in a reaction volume of 0.1 ml containing 1 mM NAD^+, 1 μg of $PGF_{2\alpha}$, and various concentration of NADPH (●) or NADH (○). (B) Type II 15-hydroxyprostaglandin dehydrogenase (17 μg) was incubated in a reaction volume of 0.1 ml with 1 mM $NADP^+$ and 1 μg of $PGF_{2\alpha}$ in the presence of varying concentrations of NADPH (○) or NADH (●). The reaction was stopped by boiling for 2 min and after dilution with 0.5 ml of Tris-HCl (0.01 M, pH 7.4), 0.1 ml was analyzed for 15-keto-$PGF_{2\alpha}$.

TABLE III: Distribution of Types I and II 15-Hydroxyprostaglandin Dehydrogenase and Prostaglandin E 9-Ketoreductase in Swine Kidney Cortex and Medulla.

Enzyme	Specific Activities[a]		Ratio of Specific Activities Cortex/ Medulla
	Cortex	Medulla	
Dehydrogenase Type I	0.11	0.01	11.0
Dehydrogenase Type II	0.07	0.12	0.6
9-Ketoreductase	0.18	0.16	1.1

[a] n moles of product generated per 10 min per mg of protein of cytoplasmic fraction.

1971; Larsson and Anggard, 1973). Thus, determination of levels of NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity in the medulla and cortex would give some estimate of the regional contamination of the medulla and cortex preparations. We found an 11:1 ratio of NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase activity in homogenates and in cytoplasmic fractions of cortex and medulla, suggesting that the two preparations were not contaminated to any great extent (Table III).

In order to demonstrate the strict coenzyme requirements of the two 15-hydroxyprostaglandin dehydrogenases, the medulla and cortex preparations were fractionated by DEAE-Sephadex chromatography. The protein and activity profiles of the type I and II 15-hydroxyprostaglandin dehydrogenases and prostaglandin E 9-ketoreductase of renal cortex and medulla are shown in Figure 1. The major peak of 15-hydroxyprostaglandin dehydrogenase activity (type II) of swine kidney medulla (Figure 1B) passed through the DEAE-Sephadex column under our experimental conditions. It had relatively strict dependence on $NADP^+$ as a cofactor and could not be separated from 9-ketoreductase activity. The second peak of 15-hydroxyprostaglandin dehydrogenase activity (type I) could have been due to cortex contamination. As shown in Figure 1A, swine kidney cortex also contained both types I and II 15-hydroxyprostaglandin dehydrogenase. The ratio of type I to type II 15-hydroxyprostaglandin dehydrogenase in cortex was about 1.5:1. Since the cortex preparation is not free of medulla contamination, some of this type II 15-hydroxyprostaglandin dehy-

drogenase activity may be of medulla origin. Prostaglandin E 9-ketoreductase activity was found in both cortex and medulla preparations and the enzyme eluted with type II 15-hydroxyprostaglandin dehydrogenase during DEAE-Sephadex chromatography (Figure 1).

We had previously shown that DEAE-Sephadex chromatography under controlled experimental conditions separated chicken heart (type I) and monkey brain (type II) 15-hydroxyprostaglandin dehydrogenases. In addition to their physical separation, they could be distinguished by their coenzyme requirements and their sensitivity to inhibition by reduced pyridine nucleotides. The cortex type I 15-hydroxyprostaglandin dehydrogenase was NAD^+ dependent and was inhibited by NADH but not by NADPH. The medulla (and cortex) type II 15-hydroxyprostaglandin dehydrogenase was $NADP^+$ dependent and was inhibited by NADPH but not NADH. These inhibitions of types I and II 15-hydroxyprostaglandin dehydrogenase by the reduced pyridine nucleotides are shown in Figure 2.

We have not been able to separate the type II 15-hydroxyprostaglandin dehydrogenase and prostaglandin E 9-ketoreductase in monkey brain and in human red blood cells, at least by DEAE-Sephadex, hydroxylapatite, or phosphocellulose chromatography (Lee and Levine, 1974b) and have suggested that type II 15-hydroxyprostaglandin dehydrogenase and prostaglandin E 9-ketoreductase may be involved in regulation of the function of PGE_2 and $PGF_{2\alpha}$ (Lee and Levine, 1974b). PGE_2 has been shown to participate in the intrarenal distribution of blood flow (McGiff and Itskovitz, 1973). PGE_2, the most abundant prostaglandin in the medulla, is also a relatively poor substrate for type II 15-hydroxyprostaglandin dehydrogenase, the major dehydrogenase in the medulla. Perhaps, this coupled NADPH-dependent conversion of PGE_2 to $PGF_{2\alpha}$ and the $NADP^+$-dependent inactivation of the product, $PGF_{2\alpha}$, serves to regulate the extent and duration of vasodilation by PGE_2 in the kidney medulla.

The distribution of types I and II 15-hydroxyprostaglandin dehydrogenases and prostaglandin E 9-ketoreductase shown in Table III was found in several preparations of medulla and cortex of swine kidneys. Experiments designed to determine the distribution of prostaglandin E 9-ketoreductase and types I and II 15-hydroxyprostaglandin dehydrogenase in the rabbit kidney are complicated by the very active prostaglandin $\Delta 13$-reductase activities. However, by measuring the production of 13,14-dihydro-15-keto-$PGF_{2\alpha}$, as well as 15-keto-$PGF_{2\alpha}$, a distribution of these activities in the medulla and cortex can be obtained. In the rabbit, the cortex has about equal amounts of type I and type II dehydrogenase. The cortex has about three times more type II dehydrogenase and prostaglandin E 9-ketoreductase than the medulla, and while we do find type I dehydrogenase in the cortex, we could not detect type I dehydrogenase activity in the medulla.

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