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STUDIES ON RAT KIDNEY 15-HYDROXY-PROSTAGLANDIN DEHYDROGENASE*

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Abstract—Rat kidney 15-hydroxy-prostaglandin dehydrogenase (PGDH) was isolated, and its characteristics and the effects of various drugs upon it were examined. The enzyme was found in the cell cytosol; was labile when unfrozen; and most active at alkaline pH, at 41°, and with the E prostaglandins. Additionally, the enzyme was inhibited by furosemide (K_i = 0.019 mM), ethacrynic acid (K_i = 0.27 mM), phenylbutazone (K_i = 0.16 mM), acetylsalicylic acid (K_i = 3.8 mM), and potassium cyanide (K_i = 1.03 mM). Inhibition of PGDH may play a role in the mechanism of action of the diuretic and anti-inflammatory drugs. Little or no inhibition was seen with amobarbital, hydralazine, alpha-methyldopa, bethanidine and guanethidine. Amobarbital inhibits NADH oxidase (K_i = 0.5 mM), but does not inhibit PGDH. This drug, therefore, may be useful in permitting the use of the fluorometric assay for PGDH in preparations of PGDH contaminated by NADH oxidase.

The significance of prostaglandins (PG)‡ in renal physiology has received much attention, including a proposed role in the etiology and pathogenesis of hypertension [1, 2]. Though most speculation concerning the role of PG has centered around alterations in PG synthesis as the primary event, alterations in PG degradation may also be important.

One of the major metabolizing enzymes for PG is PGDH. This enzyme oxidizes 15-hydroxy-PG to 15-keto-PG, resulting in a 10-fold loss of PG activity [3]. PGDH has been extensively studied in human placenta and swine and bovine lung [4]. It has also been studied in chicken heart, dog lung, and various monkey tissues [5]. The kidney enzyme has been partially purified in swine [5, 6] and localized to the kidney cortex [7, 8]. This study was initiated first to determine various kinetic parameters in the rat kidney enzyme so as to facilitate the development of a quantitative histochemical assay of PGDH in substructures of the kidney nephron. Second, the effects of various drugs on PGDH activity were studied, especially those known to have significant effects on renal function and on blood pressure regulation.

MATERIALS AND METHODS

Chemicals. NADH, NAD^+, DEAE cellulose (medium mesh 0.95 mequiv/g) and Sephadex G-100-120 were obtained from the Sigma Chemical Co., St. Louis, Mo. The PG nomenclature used is as previously referenced [7, 9]. The source of drugs is as indicated.§ All other reagents and chemicals were of analytical grade.

Enzyme assay. Spectrophotometric assays were analyzed with a Beckman DU-2 (Beckman Instrument Co., Fullerton, Calif.). Fluorometric assays were carried out using an Aminco Fluoro-Microphotometer (American Instrument Co., Silver Springs, Md.) modified for 1-cm diameter round cuvettes and a mercury arc lamp. The primary filter was No. 5860 and the secondary filters were Nos. 4303 and 3387.

Kidneys were taken from Sprague–Dawley albino rats, 250–350 g, obtained from Zivic Miller Laboratories or the Mellon Institute of Pittsburgh, Pa. The animals were maintained on Charles River CD Rat Chow and were fasted 12 hr before sacrifice.

PGDH. Spectrophotometric assays were carried out at 37° by incubating PGDH in 1 ml of 100 mM potassium phosphate buffer, pH 7.5, 2 mM ME, 2 mM EDTA, 0.77 mM NAD^+, 0.11 mM PGE_2, and 0.02% BSA. Aliquots were taken at 0, 5, 15 and 30 min of reaction. The 15-keto-PG formed was determined by the chromophore method [10]. This method was used to assay all steps in the purification procedures, in the subcellular fractionation, in the pH optimum, and at other times as indicated.

Fluorometric assay of purified PGDH was conducted in a similar manner as for the lung enzyme [11]. The reaction was carried out at 37° in 1 ml containing 100 mM potassium phosphate buffer, pH 7.5, 2 mM ME, 2 mM EDTA, 0.2 mM NAD^+ and 0.02% BSA. Sufficient PGDH was added to reduce

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‡ Abbreviations: PG = prostaglandins; PGDH = 15-hydroxy-prostaglandin dehydrogenase; ME = 2-mercaptoethanol; EDTA = ethylenediaminetetra-acetic acid; and BSA = bovine serum albumin.

§ Drug source and lot numbers: propranolol, No. 81-6474-1, Ayerst, New York, N.Y.; guanethidine sulfate, No. M-5580, hydralazine, M-962, CIBA, Summit, N.J.; alpha-methyl-dopa, No. C2983, ethacrynic acid, M-2355, and hydrochlorothiazide, Merck, Sharpe & Dohme, West Point, Pa; bethanidine sulfate, No. 43157, A. H. Robins Co., Richmond, Va.; furosemide, NDC-39-61, Hoechst Pharm., Somerville, N. J.; sodium nitroprusside dihydrate, 0002-05064, Roche Lab., Nutley, N.J.; amobarbital, No. 05D66, Eli Lilly Co., Indianapolis, Ind.; PGE_1, No. 10315-VDV-115. PGE_2, No. 5-PRC-2001A (S), PGA_1, No. 10315-VDV-97, PGA_2, No. 10981-RBJ-120, $PGF_{2\alpha}$, No. 11221-JHK-46.7, Upjohn Co., Kalamazoo, Mich.

Table 1. Isolation of PGDH of rat kidney

Isolation*	Total protein	PGDH† Sp. act. (m-moles kg^{-1} hr^{-1})	PGDH† Total act. (m-moles hr^{-1})	NADH oxidase Sp. act. (m-moles kg^{-1} hr^{-1})	NADH oxidase Total act. (m-moles hr^{-1})
(1) Whole homogenate	25.3	5.1	0.130	20,000	500
(2) 78,000 *g* Supernatant	6.15	16.1	0.099	588	3.62
(3) Ammonium sulfate	2.56	24.0	0.062	36	0.092
(4) DEAE cellulose	2.26	18.6	0.042	2.1	0.005
(5) Sephadex G-100	0.026	157	0.004	0	0

* Purification steps are as described in Materials and Methods.
† Spectrophotometric assay was used as described in Materials and Methods.

0.05 to 0.2 μM NAD^+/min. The prostaglandins were added in the amounts indicated to start the reaction. Reaction rates were calculated from the rate of change of flourescence during the first few min of reaction. The fluorometer was standardized enzymatically on each day it was used by adding sufficient NAD^+ to generate 3–6 μM NADH in 100 mM phosphate buffer, pH 7.5, 2 mM ME, 0.02% BSA, 3 μg/ml of yeast alcohol dehydrogenase and 20 mM ethanol. A stock solution of NAD^+ was standardized weekly by the spectrophotometric assay [12, 13].

NADH oxidase. NADH was assayed fluorometrically at 37° using 1 ml of a reaction mixture containing 100 mM phosphate buffer, pH 7.5, 2 mM ME, 2 mM EDTA, 0.02% BSA, and sufficient enzyme to oxidize 0.05 to 0.5 μM NADH/min. The reaction was started with 5–8 μM NADH.

Protein determinations were performed by the method of Lowry *et al.* [14]. Enzymatic activities are expressed as m-moles substrate transformed/kg of protein/hr at 37°; (m-moles kg^{-1} hr^{-1}), unless otherwise indicated.

Enzyme purification. PGDH was isolated using a modification of previously published methods [6, 8]. All steps were at 4°. The rat kidneys were homogenized in 2 vol. of 60 mM phosphate buffer, pH 7.5, 2 mM ME, and 2 mM EDTA for 3 min in a Waring blender and then for 10 strokes in a ground glass homogenizer (see Table 1). The homogenate was centrifuged first at 12,000 *g* for 15 min, then at 78,000 *g* for 30 min. The 78,000 *g* supernatant was adjusted with ammonium sulfate crystals to 30% saturation (167 g/liter) yielding a supernatant which was adjusted to 70% ammonium sulfate (448 g/liter). The 30–70% ammonium sulfate precipitate was dissolved in 10 mM phosphate buffer containing 2 mM ME, 2 mM EDTA and 1.4 μM NAD^+ and dialyzed twice for 3 hr against 200 vol. of the same buffer. The preparation was applied to a 4 × 13 cm DEAE cellulose column which was previously equilibrated with the same buffer. After washing with the buffer until the ultraviolet absorption at 280 nm had returned to the baseline, the column was eluted with a linear gradient utilizing 1500 ml of 10 mM phosphate buffer in the mixing chamber and 1500 ml of 400 mM buffer in the reservoir. Each solution contained 1.4 μM NAD^+, 2 mM EDTA, 2 mM ME, and was at pH 7.5. The PGDH was eluted from the column at 10–225 mM buffer concentration. The bulk of the NADH oxidase was found in the 225–400 mM fractions. The PGDH fractions were pooled and the protein was recovered in 70% ammonium sulfate. The preparation was next applied to a 2.5 × 100 cm Sephadex column (volume applied to column was less than 5 per cent column volume). The column was eluted with 10 mM buffer containing the ME, EDTA and NAD^+. Peak NADH oxidase activity eluted at 1.03 bed volumes. Peak PGDH activity eluted at 1.40 bed volumes. Then 1.26 to 1.94 bed volume fractions were pooled and the protein was recovered in 70% ammonium sulfate. After dialyzing 8 hr against 500 vol. of the eluent buffer, the final enzyme was assayed and stored at 5–10 mg protein/ml.

RESULTS

The isolation procedure yielded a PGDH preparation which was free of NADH oxidase activity (Table 1). When necessary, especially at the column chromatography steps, greater yields of PGDH were sacrificed in selecting the fractions to be pooled in order to insure complete removal of NADH oxidase activity.

The half time of inactivation of the enzyme upon storage was as follows: 3 hr at 37°, 3–4 days at 4°, 1 month at −20°, and about 1 year at −90°. The short half time for the inactivation of the enzyme, especially in the unfrozen state, is significant in determining the final recovery during purification. The time required for the isolation varied from 3 to 6 days.

The subcellular distribution of PGDH (whole kidney homogenized in 0.25 M sucrose) revealed that 86 per cent of the activity was in the 115,000 *g* supernatant. The remaining activity was found in the microsomal fraction. A similar distribution was previously reported for rat kidney [15].

The pH optimum for activity was found to be relatively sharp around 9.6 (Fig. 1). The ratio of activity of pH 9.6 compared to pH 7.4 was approximately 5. An overlapping of the buffer systems at the various

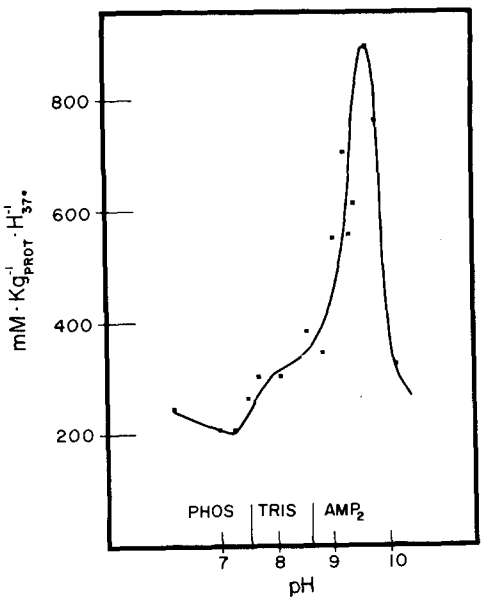

Fig. 1. Effect of pH on rat kidney PGDH. The activity of PGDH was determined spectrophotometrically by incubating purified PGDH (0.05 mg/ml) at 37° (Materials and Methods). The reactions were buffered with 100 mM sodium phosphate, Tris, or 2-amino-2-methyl-1,3-propanediol (AMP_2).

pH values revealed that the choice of buffers had no effect on enzyme activity.

The effect of temperature on PGDH is shown in Fig. 2. The highest activity was around 41° with a marked decrease in activity above and below this temperature.

The specificity of PGDH for substrates is shown in Table 2. At 120 μM PG, the reactions were linear for 30 min. PGE_2 yielded the highest reaction velocity and the lowest K_m while PGA_2 was the least active.

The effect of several drugs on PGDH activity was determined (Table 3). Amobarbital did not inhibit

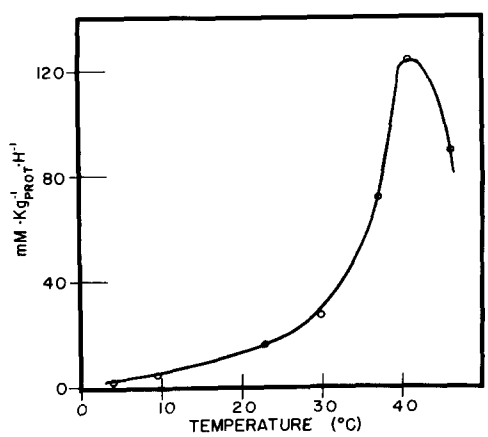

Fig. 2. Effect of temperature of kidney PGDH. The activity of PGDH was determined fluorometrically by incubating purified PGDH (0.03 mg/ml) with 44 μM PGE_2 and 0.2 mM NAD^+ at 37° (Materials and Methods). Activity was calculated from the rate of NADH formation from 0 to 15 min of reaction after correcting for the effect of temperature on fluorescence of NADH.

Table 2. Specificity of rat kidney PGDH*

Substrate	K_m (μM)	Relative V_{max}
PGE_2	0.36	100.0
PGE_1	1.00	80.6
PGA_1	8.80	61.4
$PGF_2\alpha$	1.53	60.0
PGA_2	5.13	15.6
NAD^+	8.0	

* Fluorometric assays are as described in Materials and Methods utilizing purified PGDH (0.1 mg/ml). Relative velocities were calculated with the observed K_m and the relative rate obtained with 120 μM PGE_2. The K_m for prostaglandins was determined with 200 μM NAD^+. The K_m for NAD^+ was determined with 120 μM PGE_2.

PGDH. However, over 99 per cent of NADH oxidase was inhibited by amobarbital. Potassium cyanide inhibited both enzymes. The antihypertensive drug sodium nitroprusside caused significant PGDH inhibition, but at levels much higher than what is used therapeutically. This agent has some of the chemical characteristics of a cyanide. Propranolol was an inhibitor, but at relatively high concentrations. Alphamethyldopa, bethanidine, hydralazine and guanethidine had no effect on PGDH at the levels tested. Again, these levels were much greater than one would expect under therapeutic conditions. The diuretics furosemide and ethacrynic acid were tested. Furosemide was a potent inhibitor of PGDH, and the inhibition was of the noncompetitive type. These results are similar to the findings with human placental PGDH [16]. Ethacrynic acid caused a similar inhibition; however, the concentration of ethacrynic acid required for half-maximal inhibition of PGDH was fourteen times greater than that for furosemide. The anti-inflammatory drugs phenylbutazone and acetylsalicylic acid were effective inhibitors of PGDH.

DISCUSSION

In this study, rat kidney PGDH was isolated. Some of its characteristics and the effects of various drugs upon it were examined. The enzyme was most active at oxidizing PGE_2, followed by PGE_1, PGA_1, $PGF_2\alpha$ and PGA_2. These relative rates agree more with those of the pig lung enzyme than those of the beef lung or human placental enzyme [4, 17–20]. Published ranges of the K_m values for the various substrates are: PGE_2 (5.3 to 200 μM), PGE_1 (1.14 to 7.7 μM), PGA_1 (4–14 μM) PGA_2 (6–25 μM), $PGF_2\alpha$ (6–31 μM), and NAD^+ (30–800 μM), depending upon the author and tissue [8, 17–20]. It can be seen from Table 2 that the K_m values for the substrates of the rat kidney enzyme are generally lower than those found in the other tissues.

The vasodilator and anti-adrenergic antihypertensive drugs had little or no effect upon rat kidney PGDH at relatively high drug concentrations. This suggests that PGDH may not be involved in their mechanism of action.

In contrast to the antihypertensives, the diuretic drugs furosemide and ethacrynic acid did inhibit PGDH. Both drugs are potent diuretics in dogs and man [21–23]. They along with the E prostaglandins

Table 3. Inhibitors of rat kidney PGDH and NADH oxidase

Enzyme*	Inhibitor: Drug	Inhibitor: Concn (mM)	Inhibitor: Per cent inhibition†	K_I‡ (mM)
PGDH	Amobarbital	4	0	
	Potassium cyanide			1.03
	Sodium nitroprusside	0.4	20	
	Hydralazine	1.8	0	
	Alpha-methyldopa	2.6	0	
	Bethanidine	0.32	0	
	Guanethidine	0.17	0	
	Propranolol			1.72
	Furosemide			0.019
	Ethacrynic acid			0.27
	Phenylbutazone			0.16
	Acetylsalicylic acid			3.8
NADH oxidase	Amobarbital			0.5
	Potassium cyanide			0.08

* Purified PGDH (0.1 mg/ml) was assayed fluorometrically as described in Materials and Methods with 90 μM PGE_2 and 0.7 mM NAD^+. Due to interference of fluorescence, the chromophore spectrophotometric method was used for ethacrynic acid and acetylsalicylic acid. NADH oxidase was assayed with whole kidney homogenate (Materials and Methods).

† Per cent inhibition is recorded only at the level of drug tested.

‡ Concentration for half-maximal inhibition (K_I).

have been shown to cause a redistribution of renal blood flow away from the antidiuretic juxtaglomerular and outer medullary nephrons coincident with their diuretic action [24, 25]. Rats are relatively insensitive to ethacrynic acid, yet the qualitative response is not unlike that seen with furosemide in this species [26]. In the present study, the concentration for half-maximal inhibition of PGDH by ethacrynic acid was fourteen times greater than for furosemide. It is suggested that the relative insensitivity of the rat to ethacrynic acid-induced diuresis can be explained at least in part on the basis of its decreased ability to inhibit rat kidney PGDH. Thus, one mechanism of action of these agents may involve the inhibition of PGDH, thereby prolonging the action of renal PG. This concept also lends additional support to the role of PG in renal function. Other evidence for a role of PG in the regulation of renal function comes from studies on inhibition of PG synthesis, such as that attended by alterations in renal function in dog kidney with inhibitors of PG synthesis [25]. It remains to be determined what the relative importance of PGDH inhibition is compared to other events which have been observed with some diuretics, such as inhibition of carbonic anhydrase [21] and adenosine triphosphatase [27].

Inhibition *in vitro* and *in vivo* of PG biosynthesis by the anti-inflammatory drugs acetylsalicylic acid and phenylbutazone is well documented [28]. This property of these drugs has been proposed as one of their mechanisms of action. The concentrations for half-maximal inhibition which were obtained for the inhibition of PGDH by these drugs are of the same order of magnitude as the concentration for half-maximal inhibition of PG biosynthesis. The significance of these findings is as yet unclear.

Finally, there was a lack of inhibition of PGDH by amobarbital. The well-known effect of this drug as an inhibitor of NADH oxidase was also demonstrated in this study (Table 3). Amobarbital has been routinely included in assays of various enzymes in whole tissue homogenates where NADH is a cofactor and NADH oxidase is present [29, 30]. There are several problems in the development of a histochemical assay for PGDH. The major ones are interference by NADH oxidase, a relatively high tissue blank and a relatively low PGDH activity in kidney. The lack of inhibition of PGDH by amobarbital is an important observation in this matter, since the oxidase can be nearly totally eliminated in a selective manner.

In summary, the partial purification of rat kidney PGDH was carried out to facilitate the description of some of its kinetic parameters. The enzyme may play a role in the mechanism of action of the diuretic drugs furosemide and ethacrynic acid and the anti-inflammatory drugs acetylsalicylic acid and phenylbutazone. Amobarbital, which inhibits NADH oxidase, does not inhibit PGDH and, therefore, may aid in the development of a sensitive histochemical assay for PGDH.

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