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RAT RENAL MEDULLA POSSESS HIGH CAPACITY TO CATABOLIZE PROSTAGLANDINS

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Summary.Prostaglandin E_2 is converted to 15-keto-13,14 dihydro prostaglandin E_2,15-keto-prostaglandin $F_{2\alpha}$ and 15-keto-13,14 dihydro prostaglandin $F_{2\alpha}$,by supernatants from rat kidney medulla.The main pathway for prostaglandin E_2 inactivation is the combined action of 15 hydroxy dehydrogenase and $\Delta 13$ reductase enzymes.9-Keto-reductase route constitutes a minor pathway. Prostaglandin $F_{2\alpha}$ is converted into 15-keto-prostaglandin $F_{2\alpha}$,15-keto-13,14 dihydro prostaglandin $F_{2\alpha}$ and 15-keto-dihydro prostaglandin E_2.Enzyme activities are time and substrate-concentration dependent.In the presence of an excess of substrate,rat renal medulla inactivates 40 and 56 times more prostaglandin E_2 and prostaglandin $F_{2\alpha}$,respectively,than the amount which is released under basal conditions.These results are in contrast to the generally accepted concept that the kidney cortex is the sole site of renal prostaglandin catabolism,and suggest,for the first time,that rat renal medulla may be a key site for the modulation of prostaglandin levels in the kidney.

Introduction.Renal PGs oppose to the excessive action of the salt and water conserving system (1).Under stress conditions,PGE_2 is the principal intrarrenal mediator that attenuates the renal vasoconstrictor and antidiuretic effects of angiotensins,vasopresin and alpha adrenergic nervous activity.Zonal redistribution of renal blood flow is also mediated by PGE_2 (2).However,zonal stratification within the kidney of the enzymes and cofactors that synthetized and metabolized PGs,had been considered major determinants of their local physiological roles.Most available information demonstrates that the main site of renal PG catabolism,is the cortex (3).However,studies performed in other species than the rat,(with the exception of the swine),described a distinct degradative ac-

Abbreviations used in the text,are:PG,prostaglandin;PGE_2,prostaglandin E_2; 15KDH-PGE_2,15-keto-13,14 dihydro prostaglandin E_2;15K-$PGF_{2\alpha}$,15-keto-prostaglandin $F_{2\alpha}$;15KDH-$PGF_{2\alpha}$,15-keto-13,14 dihydro prostaglandin $F_{2\alpha}$;$PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$;15-PGDH,15 hydroxy dehydrogenase;13-PGR, $\Delta 13$ reductase; 9K-R,9-keto-reductase;15K-PGE_2,15-keto-prostaglandin E_2;15K-$PGF_{2\alpha}$,15-keto-prostaglandin $F_{2\alpha}$;9-PGDH,9 hydroxy dehydrogenase.

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tivity in the renal medulla (4).Available data for the rat,under physiological or pathological conditions,were performed using whole kidney and cortex homogenates,without any zonal stratification study (5-9).All this information induced to the generally accepted concept that renal cortex is the sole site of PG degradation from renal origin.Hence,the high levels of PGs synthetized by papilla and medulla,would be inactivated in cortex.In contrast,here we demonstrate that rat renal medulla possess high capacity to catabolize PGE_2 and $PGF_{2\alpha}$,which is enough to inactivate the PG synthetized by itself as well as that arising from the papilla.

Materials. [1-^{14}C]-PGE_2 (41.0 mCi/mmol) and [1-^{14}C]-$PGF_{2\alpha}$ (40.0 mCi/mmol), were purchased from New England Nuclear (Boston,MA).Authentic standards for PGs were the generous gift of Dr.John Pike,of the Upjohn Corp.(Kalamazoo,MI). Silica Gel G plates 0.25 mm thick,from E.Merk,A.G.,Germany.X-ray film for autoradiography,from Eatsman Kodak Co.(Rochester,N.Y.).PGE_2 and $PGF_{2\alpha}$ antiserum,from Pasteur Institute,Paris.All other chemicals and solvents were of reagent grade.

Methods.Assay for PG synthetase.After decapitation of male Wistar rats (250-280 g),both kidneys were removed and maintained on ice cold Krebs' solution. Each kidney was cut in half through the pelvis along its longitudinal axis, and the renal cortex,medulla and papilla were separated by scissor and scalpel dissection.This procedure provide uniform and defined regions (3,10).The tissue was weighed to the nearest 10 mg on an analitical balance.Tissue slices were then incubated in a Dubnoff metabolic shaker bath at 37ºC in flasks containing 2 ml of Krebs Ringer bicarbonate buffer,with 5.5 mM of glucose, under an atmosphere of 95% O_2-5% CO_2.After 30 minutes,the supernate was removed with Pasteur pipete,acidified to pH 3 with 1 M citric acid,and extracted three times with two volumes of chloroform.The combined organic phases were evaporated under a stream of nitrogen.The recovery of the extraction procedure was about 95%-98%.The residues were dissolved in 0.1 M buffer phosphate pH 7.4.One alicuot of the solution was used for cuantitation by radioimmuno assay (11).The results were compared by means of the Student t test,and differences were considered statistically significant when p values were less than 0.05.

Assay for PG degradative enzymes.The kidneys were dissected as we previously described,washed with ice cold 0.05 M KH_2PO_4-NaOH buffer (pH 7.4) and immediately homogenized in a Polytron tissue homogenizer (top speed,10 secons),in four volumes of the same buffer at 0ºC.PG catabolizing enzymes were measured in a supernatant occurred at constant rate for 15 minutes at 20.000 g.The method for determining PG degradative enzymes was adapted from that of Pace-Asciak et al (13).Aliquots of supernatant from medulla,were incubated for differents periods of time at 37ºC,with the addition of 200 pmol of [1-^{14}C]-PGE_2 (41.0 mCi/mmol),or 1-^{14}C-$PGF_{2\alpha}$ (40.0 mCi/mmol),and known amount of cold PGE_2 or $PGF_{2\alpha}$,as correspond,in a total volume of 200 µl each incubation.The reaction was terminated by the addition of 1 M citric acid to pH 3,and extracted three times with two volumes of chloroform.The organic phase was removed and dried under stream of nitrogen.The residue was redissolved in 50 µl of chloroform:methanol (1:2,v/v),and applied to a silica gel G thin layer plate,which was developed twice in ethyl acetate/iso octane/acetic acid/water,(66:30:12: 60,v/v),upper phase),as the solvent system.Authentic standards of $PGF_{2\alpha}$ (Rf=.27), PGE_2 (Rf=.42), 15K-$PGF_{2\alpha}$ (Rf=.45), 15KDH-$PGF_{2\alpha}$ (Rf=.47), 15K-PGE_2 (Rf=.65),and 15KDH-PGE_2 (Rf=.69),were co-chromatografied and visualized by spraying the corresponding areas of the plate with 10% solution of phosphomolibdic acid in

ethanol,and heating at 110ºC.Radioactivity from the thin layer plate zones of the specific areas were determined by autoradiography,scraped off the plate and quantitated by counting in a liquid scintillation counter,with toluene-omnifluor .4% mixture.Under the conditions used,non-enzymic decomposition was negligible.Product formation was calculated from the amount of radioactivity present in zones migrating on thin layer chromatograms,and the knowledge of the substrate pool size used in each tube,with the correction of recovery for extraction and separation.All the values are the mean of five experiments,and are expressed as the mean ± S.E.Statistical significance evaluated by non paired Student's t test, and protein concentration by the method of Lowry et al (12).

Results.PG biosynthesis in rat renal medulla.The incubation of rat renal medullary slices under basal conditions,without the addition of any cofactor,results in the production of considerable amounts of PGE_2 and $PGF_{2\alpha}$ (Fig.1).The rate of PGE_2 synthesis appears to be significantly higher than $PGF_{2\alpha}$.The specific activity for PGE_2 and $PGF_{2\alpha}$ were 0.332 ± 0.0306 and 0.0372 ± 0.0073 pmol. mg $prot^{-1}$. min^{-1},respectively.

Time course of PGE_2 and PGF_2 catabolism.PGE_2 is converted into less polar and chromatographycally well resolved products after incubation with supernatant of rat renal medulla (Fig. 1A) .Metabolite 1 is identified as 15KDH-PGE_2,and represents the combined actions of 15-PGDH and 13-PGR (Fig. 4) .No 15K-PGE_2 is observed in this experimental condition,indicating a very efficient transformation into 15KDH-PGE_2.Metabolite 2 is identified as 15KDH-$PGF_{2\alpha}$.

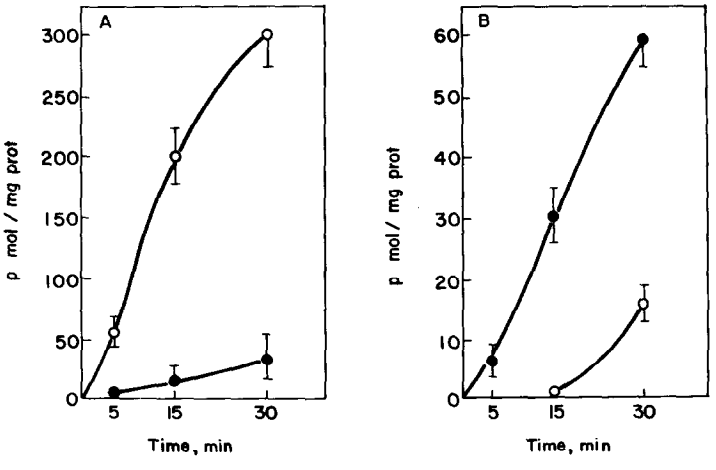


Fig.1 Convertion rate of PGE_2 (A) and $PGF_{2\alpha}$ (B) by rat renal medulla supernatant.The incubation system consisted of supernatant,radiolabeled PGE_2 (A) or $PGF_{2\alpha}$ (B),and known amount (0.25 μg) of cold PGE_2 (A) or $PGF_{2\alpha}$ (B).Each point represent the mean of 5 experiments.Results are expressed as pmol of product formed per mg of protein.

○——○ Metabolite 1 ●——● Metabolite 2

Since we use PGE_2 as substrate,the presence of metabolite 2 involved the combined action of 9K-R,15-PGDH and 13-PGR enzymes (Fig. 4).

At 5 minutes incubations,12% of the added PGE_2 is converted in metabolite 1, with no metabolite 2.At 15 minutes,metabolite 1 represent 43%,and metabolite 2,3%.At 30 minutes,60% of the added PGE_2 is converted in metabolite 1,and 6% in metabolite 2.

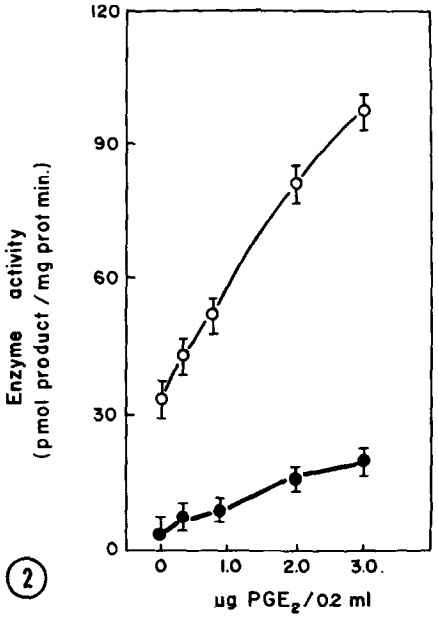
Dehydrogenase specific activity was 12.32 ± 1,26 pmol.mg $prot^{-1}$. min^{-1},and 9K-R specific activity was 0.71 ± 0.25 pmol.mg $prot^{-1}$. min^{-1},indicating that dehydrogenase pathway is the main route of PGE_2 inactivation,in our experimental conditions.

Using $PGF_{2\alpha}$ as substrate,we observed an increased transformation into metabolite 2,reaching 22% at 30 minutes of incubation (Fig. 1B) .No metabolite 1 is observed at 5 and 15 minutes of incubation,reaching 6% at 30 minutes.

The presence of metabolite 1 in this set of experiments,demonstrates 9-PGDH activity,which converts15KDH-$PGF_{2\alpha}$ in 15KDH-PGE_2 (14).Probably,some of the metabolite 1 present at 30 minutes,using PGE_2 as substrate (Fig. 1A) ,rise from the activity of this enzyme,which implicates 9K-R pathway.The specific activity for $PGF_{2\alpha}$ dehydrogenase was 2.09 ± 0.26 pmol.mg $prot^{-1}$.min^{-1},significantly lower than the rate of PGE_2 catabolism.

Influence of increasing substrate concentration on PGE_2 and $PGF_{2\alpha}$ catabolism.Fig. 2 shows the activity profiles for the catabolizing enzymes as a function of increasing concentration of substrate.It has been chosen 10 minutes of incubation,in order to define the presence of 15KDH-PGE_2 as the product of the combined action of 15-PGDH and 13-PGR,with no implication of the 9-PGDH activity (Fig 1B and Fig. 4) .The 15-PGDH activity is accounted by the presence of 15KDH-PGE_2.No significant amount of 15K-PGE_2 is observed at any concentration.On the other hand,9K-R activity is represented by the sum of 15K-$PGF_{2\alpha}$ and 15KDH-$PGF_{2\alpha}$.

The profile of $PGF_{2\alpha}$ catabolism,in the presence of excess of substrate,is shown in Fig. 3.The incubation was performed for 30 minutes,in order to define the complete sequence of reactions (Fig. 1B) .PG dehydrogenase activity (represen-



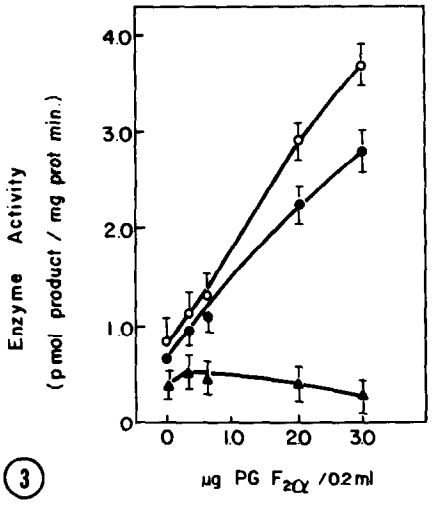


Fig.2 Effect of increasing PGE_2 concentration on 15-PGDH activity (○—○) and 9K-R activity (●—●), in rat renal medulla supernatant. The incubations conditions are the same as in Fig.1 A, except that tracer [1-^{14}C]-PGE_2 was diluted with 5 different concentrations of unlabeled PGE_2, and the period of time was 10 minutes. Enzyme activity represent the pmol of product formed per mg of protein. Mean value ± S.D of 5 experiments is represented in each point.

Fig.3 Effect of increasing $PGF_{2\alpha}$ concentration on 15-PGDH activity (○—○), 13-PGR activity (●—●), and 9-PGDH activity (▲—▲), in rat renal medulla. Each point represent the mean of 5 experiments. Incubation conditions are the same as in Fig.3 A, except that tracer [1-^{14}C]-$PGF_{2\alpha}$ was diluted with different concentrations of unlabeled $PGF_{2\alpha}$, and the period of time was 30 minutes. Calculation was described in Fig. 2.

ted by the sum of 15K-$PGF_{2\alpha}$, 15KDH-$PGF_{2\alpha}$, and 15KDH-PGE_2), increase with the amount of substrate within the range of concentration of our experimental conditions. Some substrate inhibition is observed for 9-PGDH activity. This inhibition is likely due to 15KDH-$PGF_{2\alpha}$, which accumulates during incubation and acts as substrate for the 9-PGDH enzyme.

Discussion. The present information clearly indicates that rat renal medulla possess a high rate of PG inactivation. This result is opposite with the concept that renal cortex is the sole site of PG catabolism (3). This finding is particularly important in the rat, because PGE_2, the major PG of renal origin, constricts the renal vasculature (16,17), in contrast with other species (18), and potentiates the vascular response of kidney to the vasoconstrictor effect of nerve stimulation (19) and norepinephrine infusion.

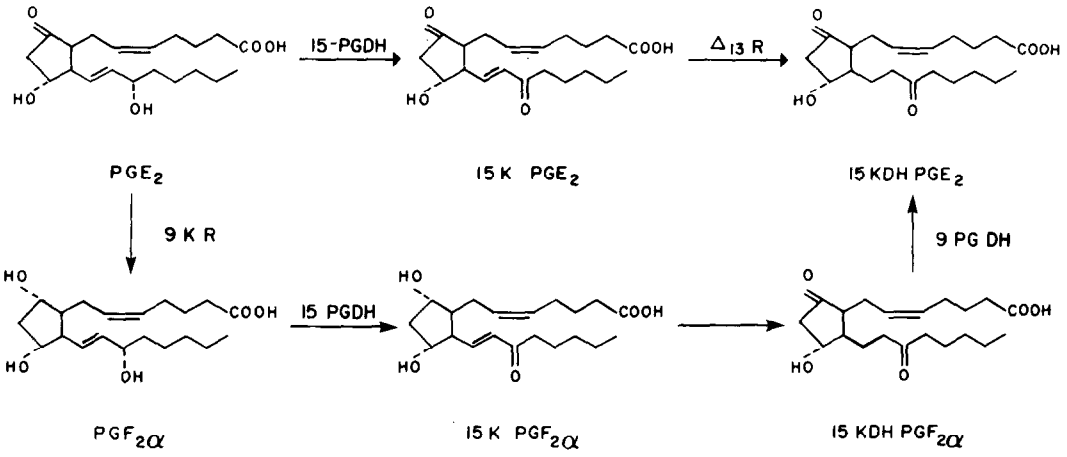


Fig.4 Sequence of steps in the catabolism of PGE_2 and $PGF_{2\alpha}$ of rat renal medulla.

These local actions of PGE_2 makes necessary its rapid catabolism to establish normal kidney function when,under conditions of stress or hormonal stimulation, a great amount of PGs are produced.

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