Renal Prostaglandin Synthesis in the Spontaneously Hypertensive Rat

MICHAEL J. DUNN with the technical assistance of DOUGLAS HOWE and MARIETTA HARRISON

From the Division of Nephrology, Department of Medicine, University of Vermont, Burlington, Vermont 05401

ABSTRACT The precise role of the kidney in spontaneous experimental hypertension is unknown. We have analyzed the rates of renal prostaglandin synthesis by utilizing a spontaneously hypertensive rat model. The synthetic rate of prostaglandin E₂, prostaglandin F₂α, and prostaglandin A₂-like products was measured in vitro with renal microsomes. In the rabbit and rat there is a steep gradient of microsomal prostaglandin synthetase from papilla to cortex with highest activities in the papilla. Comparison of the activity of prostaglandin synthetase in medullary microsomes from normotensive and hypertensive rats showed accelerated synthesis in the spontaneously hypertensive rat. These differences appeared after several months of age, were statistically significant from 3 mo of age and, on the average, represented at least a twofold increase in vitro activity.

All classes of prostaglandins were involved with increased synthesis of prostaglandin E₂, prostaglandin F₂α, and prostaglandin A₂-like material. These data reinforce and extend previous work showing alterations of granularity and presumably prostaglandin synthesis in renal medullary interstitial cells in various experimental hypertensions.

We also measured renal tissue content of prostaglandin E and prostaglandin A-prostaglandin B by radioimmunoassay. Swift and careful handling of the tissue was necessary to avoid extensive postmortem synthesis of prostaglandins. In rapidly-frozen medullary tissue only prostaglandin E was detectable in concentrations ranging from 10 to 200 pg/mg tissue. No significant differences were found in the medullary content of prostaglandin E in the control and hypertensive rats despite the increased rates of enzymatic synthesis.

We conclude that renal prostaglandin synthesis is increased in renal medullary microsomes obtained from spontaneously hypertensive rat. This apparently occurs in response to the progressive development of hypertension since young animals did not show an increase. Renal tissue prostaglandin E content did not increase and therefore appears to be a poor index of enhanced prostaglandin synthesis.

INTRODUCTION

There is a growing awareness and interest in the vasodepressor role of the kidney (1-4). The compounds which seem most likely to be mediators of an antihypertensive function are the renal medullary prostaglandins and a neutral lipid discovered by Muirhead (1). Considerably more is known about the renal prostaglandins, although their importance in physiologic situations is unknown. Whereas some authors have speculated that renal prostaglandin deficiency may cause hypertension, others have concluded that enhanced prostaglandin production may occur in response to hypertension (3). The major renal prostaglandin which could serve an antihypertensive function is prostaglandin E₂ (PGE₂) since it is vasodilatory and natriuretic in most species (2). Although PGA also shares these pharmacologic properties (4), there is considerable disagreement about the physiologic importance and biochemical origin of PGA (5, 6). Previous publications have asserted that renal prostaglandin secretion increases in experimental hypertension (3,

1 Abbreviations used in this paper: PGA, prostaglandin A; PGB, prostaglandin B; PGE, prostaglandin E; SHR, spontaneously hypertensive rat; TLC, thin-layer chromatography; WKy, Wistar Kyoto control.
that prostaglandin production by the kidney decreases in hypertension (10–12). Although these authors have utilized many different experimental models, the more important problem has been the complexity and diversity of the methodologies for the measurement of prostaglandin levels. Additionally since tissue content of prostaglandins may not accurately reflect in vivo synthetic rates, it becomes imperative to assess rates of synthesis as well as tissue levels.

The objective of the present experiments were threefold: (a) To utilize the spontaneously hypertensive rat (SHR) of Okamoto since it is a well-studied model of essential hypertension not requiring any manipulations of renal vessels or parenchyma; (b) To measure prostaglandin synthesis in vitro by using renal medullary microsomes and a controlled radiochemical assay; (c) To measure renal prostaglandin content by radioimmunoassay, taking care to minimize postmortem prostaglandin synthesis in the tissue.

As a result of these experiments we conclude that renal medullary prostaglandin synthesis (PGEs and PGFws) increases substantially as hypertension develops in the SHR.

**METHODS**

**Animals.** The rats were obtained from the University of Vermont Colony of Wistar Kyoto control (WKy) and SHR. This colony has been inbred with brother-sister matings from original breeding stock obtained from Dr. Carl Hansen, Chief, Genetics Unit, Animal Production Section, National Institutes of Health. This stock was originally derived from Okamoto-Aoki stock from Kyoto, Japan. There were very few spontaneous deaths in the SHR between 1 mo and 1 yr of age, and the occasional death which occurred was due to respiratory infections which also affected the WKy. Systolic blood pressures were recorded by tail cuff sphygmomanometry after warming the animal. Ten recordings were made and the mean of the last five pressures (systolic) were used. Pressures were recorded monthly beginning at 6 wk of age. The rats were sacrificed by decapitation. For the assay of prostaglandin synthetase the kidneys were decapsulated, cut in half sagitally, and the medulla (including papilla) of each was dissected out. The medullae from both kidneys of a single rat were paired and treated experimentally as a single sample for the synthetase assay. The wet weights of the medullae were 293±18 mg (mean±SEM) for WKy and 262±14 mg for SHR. The yield of microsomal protein from each mg of renal medulla was similar in WKy and SHR and was 0.47 and 0.42%, respectively. The tissue was chilled at all times after sacrifice of the animal. Experiments were always done by pairing a WKy and SHR of similar age for sacrifice on the same day. The animals were not paired by sex.

For the analysis of tissue prostaglandin content one kidney was rapidly removed, cut sagitally, the medulla dissected and plunged into liquid nitrogen within 1 min of sacrifice. The contralateral kidney was dissected similarly and the fresh medulla was maintained on ice for 30 min before extraction. Initial experiments showed that freezing the entire kidney and dissection of the medulla on dry ice, which is difficult, yielded PGE concentrations similar to those obtained by freezing the medulla after dissection.

**Tissue extraction.** The medullary tissue for the synthetase assay was minced with a razor blade, placed in 3-mL M Tris, pH 8.0 buffer in a Potter-Elvehejm homogenizer and homogenized vigorously. The homogenate was centrifuged at 10,000 g for 10 min at 4°C. The supernate was removed and recentrifuged at 110,000 g for 1 h at 4°C. The pellet containing the microsomes of renal medulla was resuspended in 1.0 mL of 0.05 M Tris pH 8.0, frozen over-night, and utilized the following day. The supernatant solution, from the ultracentrifugation, was boiled, recentrifuged briefly at 10,000 g, and used in the assay (13).

**Tissue for prostaglandin radioimmunoassay was placed in 3 mL methanol: water (2:1) with indomethacin 5 µg/mL and 1,000 cpn H² PGE (tracer for calculation of recoveries). Homogenization was done with a Potter-Elvehejm apparatus. The homogenate was acidified to pH 3.5 with 0.1 M HCl and extracted twice with 4 mL chloroform. The organic extract was evaporated under nitrogen and the residue was resolubilized in benzene: ethylacetate: methanol, 60:40:2, and applied to silicic acid columns (see below).**

**Synthetase assay.** The radiochemical assay of renal microsomal prostaglandin synthetase was done by using [³⁵Cl]arachidonic acid as substrate and thin-layer chromatography for separation of end products (14). The reaction mixture of 2 mL contained: microsomal suspension 0.4 mL, [³⁵Cl]arachidonic acid 0.1 µCi (Amersham/Searle Corp., Arlington Heights, Ill., sp act 38 mCi/mmol), unlabeled arachidonic acid 1 µM (Sigma Chemical Co., St. Louis, Mo.), bovine serum albumin 1 mg, glucose 2 mM, glutathione 2 mM, epinephrine 2.5 mM, hemoglobin 1 µM, and 0.4 mL of Tris buffer pH 8.0, 0.05 M (14, 15). Controls were run for each experiment with either boiled microsomes or 0.4 mL Tris buffer. Both yielded similar results with less than 3% nonenzymatic conversion of arachidonic acid to other products. Measured consumption of arachidonic acid substrate did not exceed 40% and usually was 15–25% with rat renal microsomes. The microsomal protein concentration in the reaction media was 0.5–1.0 mg/mL and was always comparable for WKy controls and the SHR.

The microsomes were incubated at 38°C for 15 min in a shakerbath. The reaction was stopped by placing the flasks in ice and adding 15 mL of chloroform-methanol, 2:1.0, 10–15 mL of chloroform-methanol, 1 mL, and 30–40 mL of distilled water. Approximately 5 mL of unlabeled carrier PGEs, PGEs, and PGF2 were added to each flask to facilitate the separation and identification of the labeled end products.

**Extraction and chromatography.** The reaction vessels were treated with the chloroform-methanol for 30 min at room temperature. The samples were then filtered (Whatman no. 1) into 125 mL separatory funnels and the flasks and filters were rinsed twice with 3 mL of chloroform-methanol 2:1. The filtrate was acidified with 4 mL of 0.03 M HCl and shaken vigorously for extraction of the prostaglandins. The aqueous layer was discarded and the organic layer was washed once with 5 mL of distilled water. The organic phase was evaporated under nitrogen and resuspended in 100 µL of chloroform-methanol, 1:1, for chromatography. Thin-layer chromatography was done by using silica gel, 20 × 20 cm precoated plastic sheets (Brinkmann Sil-G-Hy, Brinkmann Instruments, Inc., Westbury, N. Y.). The plates were spotted with 25 µL of the extracted end products and with appropriate standards (PGEs, PGEs, and PGFws were always used whereas PGB2 and PGD2 were occasionally utilized as additional standards). The plates were developed to 15 cm in a chloroform, methanol,
The radioimmunoassay utilized antibodies to PGB1 (Clinical Assays Inc., Cambridge, Mass.), H° PGB1, and cold PGB1. After 1 h incubation at 37°C, 1 ml of dextran coated charcoal, 12 mg/ml, was added for 3 min followed by centrifugation to separate bound and free PGB. The supernatant fraction was counted in a liquid scintillation spectrometer. A column blank composed of solvents without tissue was run for every experiment and the value (always less than 30 pg PGB) subtracted from all tissue concentrations. The sensitivity of the standard curve was 50% displacement at 200 pg of PGB and detection limits varied between 15 and 30 pg. These methods cannot differentiate between PGA and PGB in the original sample. No attempt was made to discriminate between PGE1 and PGE2 and therefore the values for PGE in Table I are reported without designation of the unsaturation of the side chain. Since the kidney predominantly synthesizes PGE1 it is probable that most or all of the measured PGE was PGE1. Proof that we were indeed measuring prostaglandins and not nonspecific binding of other substances is presented elsewhere; namely that indomethacin in vivo reduced tissue levels 75% and that indomethacin or meclofenamate reduced renal medially cell culture production of PGE by 90% (17) as measured by this radioimmunoassay.

**Statistics.** We utilized nonparametric and nonpaired analyses, namely the Mann-Whitney U test. Although the experiments were paired for age and day of sacrifice and assay, they were not paired by sex. The differences observed for PG synthetase gained even greater significance with a paired analysis (Wilcoxon matched-pairs signed-ranks test), whereas the differences observed in prostaglandin content of tissue were not significantly different with either paired or nonpaired analysis.

**RESULTS**

The **TLC system.** Fig. 1 shows the separation of the various prostaglandins on our TLC system. The migration pattern of the unlabeled standards, as determined from iodine staining, is superimposed on the autoradiograph for comparison. There is clearcut separation of PGF2α and PGE. The labeled end product which comigrates with the PGα standard may be 15 keto-dihydro PGE2 or hydroxy acids rather than authentic PGα (see Discussion). It is also apparent that indomethacin, 5 μg/ml, significantly reduced synthetase activity on the autoradiogram. By using rabbit medulla, indomethacin or meclofenamate 5 μg/ml, inhibited 93 to 99% of synthetase activity in vivo.

**Distribution of prostaglandin synthetase.** We evaluated the distribution of prostaglandin synthetase in the rat and rabbit kidney. In agreement with other workers (19), we found a preponderance of prostaglandin synthetase in the papilla with a decreasing amount proceeding through medulla to cortex. The ratio of synthetase activity (Eα and Fα) between papilla and cortex was approximately 100 to 1. It was also noted that the activity of prostaglandin synthetase was greater in the rabbit especially in the deeper zones of the kidney. In subsequent studies of hypertensive rats and their controls, we utilized the medulla and papilla for micro-

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<th>Age</th>
<th>Frozen samples*</th>
<th>Fresh samples†</th>
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<td>WKY (6)</td>
<td>SHR (6)</td>
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<tr>
<td>2</td>
<td>149 ± 38</td>
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<td>4–5</td>
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<td>60 ± 26</td>
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* Frozen in liquid nitrogen within 60 s of slaughter.
† Contralateral kidney kept at 4°C, on ice, for 30 min.
‡ Systolic blood pressure for the four ages respectively: 153 ± 4, 123 ± 4, 131 ± 6, and 123 ± 3 mm Hg, mean ± SEM. The 2 mo ages may be aritfactually high due to the small size of the tail.
§ n = 6 at each age. Systolic blood pressure for the four ages respectively was: 175 ± 4, 194 ± 4, 209 ± 8, and 186 ± 5 mm Hg, mean ± SEM.

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**Table I**

**Levels of PGE in Rat Renal Medulla**

<table>
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<th>Age</th>
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The weights of the fresh medullae were similar in the WKy (293±18 mg) and the SHR (262±14 mg). If the activity of the prostaglandin synthetase was expressed per milligram of medullary weight the differences between WKy and SHR would be increased, albeit slightly. Also, the microsomal yields are similar between the two groups of rats, 0.47% for WKy and 0.42% for SHR. Since medul-

\[ \text{protein} \]
The values are expressed per milligram of microsomal protein derived from renal medulla (including papilla). Results are given as the mean±SEM and the number of animals studied is indicated. All animals were paired by age and day of experiment, but not by sex. The age at the time of sacrifice is indicated in months. Statistical analyses, comparing WKy and SHR of the same ages, were done with the Mann-Whitney U test; * = P < 0.05; ** = P < 0.02; *** = P < 0.01. The apparent decline of synthetase activity in both species at 6-7 mo may be artifactual (see text).

In a single experiment we assessed the differences of PGF synthetase in microsomes from a WKy control and a SHR (6 mo of age) comparing outer cortex, medulla, and papilla. When these zones of the kidney were analyzed separately, the increased prostaglandin synthetase activity in the SHR as compared to the WKy was discernible in the papilla and medulla but not in the cortex.

Prostaglandin levels in renal tissue. The data in Fig. 6 emphasize the necessity of rapid freezing and careful handling of the tissue. Postmortem synthesis of PGF is rapid and substantial. Whereas base-line levels, at 45 s, were less than 100 pg/mg tissue, the amount of PGF increased rapidly especially in papilla, within 10-15 min after decapitation of the rat. The amounts of PGA (including PGB) were considerably less at all points of time. Because of these results we froze tissue samples in liquid nitrogen within 60 s of slaughter and homogenized the tissue in solvents containing indomethacin. For comparison we also analyzed tissue kept on ice for 30 min after slaughter (subsequently called "fresh"). Table I summarizes these data for renal medullae (including papillae) of six WKy rats and six SHR sacri-

4-wk period, and in several experiments the microsomes were frozen for several days rather than overnight. The results for PGF synthetase are shown in Fig. 4 and the pattern closely resembles the results with PGE synthetase. The hypertensive rats showed a progressive in-

The results for PGE syn-

Figure 3 Renal medullary PGE synthetase in WKy and SHR at five different ages. The ordinate depicts the pmol of PGE synthesized in vitro during a 15-min incubation. The values are expressed per milligram of microsomal protein derived from renal medulla (including papilla). Results are given as the mean±SEM and the number of animals studied is indicated. All animals were paired by age and day of experiment, but not by sex. The age at the time of sacrifice is indicated in months. Statistical analyses, comparing WKy and SHR of the same ages, were done with the Mann-Whitney U test; * = P < 0.05; ** = P < 0.02; *** = P < 0.01. The apparent decline of synthetase activity in both species at 6-7 mo may be artifactual (see text).

Figure 4 Renal medullary PGF synthetase in WKy and SHR at five different ages. Refer to legend for Fig. 3 for details.
fixed at each of four different ages. The data presented are only for PGE since PGA determinations in 28 renal medullae were never distinguishable from zero. There are no significant differences between the PGE values whether analyzed in a nonpaired or paired fashion in normotensive and hypertensive renal medullae. Likewise there are no significant differences between WKy and SHR when the "fresh" medullae were analyzed. "Fresh" PGE levels were always higher than "frozen" concentrations, as was predicted by the aforementioned studies (Fig. 6). The "fresh" tissue was iced (±4°C), and hence showed lower PGE content than the tissue maintained at room temperature (Fig. 6).

Cortical content of PGE and PGA was unmeasurable in eight frozen and eight fresh renal specimens from four WKy and four SHR. 17 pairs of WKy and SHR, matched for age, had 24-h urine collections for sodium while eating a standard chow diet. There were no differences in total urine Na between the normotensive and hypertensive rats.

DISCUSSION

We have used the Okamoto strain of spontaneously hypertensive rat as an hypertensive model to look for alterations of renal prostaglandin synthesis in one type of experimental essential hypertension (21). This strain of SHR has many features in common with other strains of spontaneously hypertensive rats as well as with human essential hypertension (22). The results of sequential assays of prostaglandin synthetase, in rats of different ages, suggests that renal medullary (microsomal) prostaglandin synthesis increases as hypertension develops perhaps as an homeostatic response to physiologic alterations secondary to the hypertension. It does not seem that renal prostaglandin deficiency can explain the hypertension in this model. Based on our data, renal tissue levels of PGE in snap-frozen medullae appear to be unreliable indicators of increased total synthetic capacity for prostaglandins in the kidney.

Explanation is required for the similar content of PGE in renal medullae from WKy and SHR despite increased microsomal prostaglandin synthetase activity in SHR. There is general agreement that prostaglandins are not stored within cells (23-26). Prostaglandin synthesis is rapid, apparently limited by substrate availability and hence turnover is extensive. If precautions are taken to minimize postmortem synthesis of prostaglandins, tissue levels of PGE are very low by using our assay. These precautions may explain why the values in Table I are much lower than previously reported (5). Therefore if appropriate care is taken to avoid significant postmortem tissue synthesis, tissue levels are quite low and may be a poor reflection of in vivo synthesis (23).

Our data regarding the distribution of the synthetase system within the kidney confirms the earlier observations of others (5, 19) in the rabbit. Our results show a steep gradient of synthetase activity from papilla to cortex emphasizing the necessity to separate medulla from cortex when renal levels of synthetase are measured. The activity of the prostaglandin synthetase in rat papilla and medulla is less than in rabbit kidney papilla and medulla.

Increased synthesis of renal PGEs in vivo, could possibly modulate the blood pressure and thereby minimize its deleterious effects on the renal vasculature through the vasodilatory and natriuretic properties of PGEs (6). The potential actions of enhanced PGF2α synthesis are more difficult to assess for several reasons. The intrarenal actions of PGF2α in the dog may be to increase sodium excretion with minimal effects on blood flow but the doses required are higher than those of PGEs or PGAα and its physiologic role is unknown (27, 28). Finally, it should be stressed that the ratios of PGEs to PGF2α produced in vitro are partially dependent on the relative concentrations of arachidonic acid and of co-factors and therefore we do not know if the same

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ratios of PGE$_2$ to PGF$_{2\alpha}$ would be found in vivo (29–31). Like others (30, 31) we have also detected the formation of what appears to be PGD$_3$ (see Fig. 1) by renal microsomes.

Previously, other investigators have concluded that there was increased renal prostaglandin synthesis in response to diverse types of hypertension. Tobian and co-workers have demonstrated decreased osmophic granularity in the renal medullary interstitial cells of rats with postsalt (32), Goldblatt (33), and post-Goldblatt (34) forms of hypertension. Muehrcke et al. made similar observations in rats with desoxycorticosterone acetate-salt hypertension (35). Mandel and co-workers have reported similar findings in the species used for the present study, namely the Japanese SHR (36, 37). These groups have concluded that decreased granularity was a measure of depleted lipid precursors of prostaglandin production and therefore reflected increased synthesis. Tobian and Azar supported this conclusion by showing enhanced PGE$_2$ release in vitro from slices of medullae of hypertensive rats (38). Recent work has re-enforced these conclusions based on interstitial cell granularity by correlating decreased renal content of PGF$_{2\alpha}$ and increased granularity of renal medullary interstitial cells in rabbits after indomethacin administration (39). Sirois and Gagnon (12), who used Goldblatt and SHR models, have reported contrasting results to those of Tobian and the present report. They observed decreased release of PGE$_2$-like material from the renal papilla obtained from the hypertensive animals and incubated for 30 min in Krebs solution. Two other groups have also reported decreased renal PGE$_2$ synthesis in renal models of hypertension (10, 11).

Other workers who have used various bioassays have reported increased tissue levels of PGE$_2$-like and PGF$_{2\alpha}$-like material in the kidneys of rabbits (8) and rats (7, 40) with Goldblatt hypertension. Zusman et al. by using a RIA for PGF$_{2\alpha}$, found greater amounts of PGF$_{2\alpha}$ in the whole kidneys (unfrozen) of SHR compared to normal wistar rats (9). These results are difficult to interpret since we were unable to detect PGA in SHR renal tissue using different methodology. Grodzinska et al. (41) have published measurements of prostaglandin synthetase on renal cellular extracts of hypertensive animals (uninephrectomized rats with aortic coarctation). They did not separate microsomes from cytosol and the production of prostaglandin end products was measured by bioassay. They noted an initial fall of prostaglandin synthetase at 4 days with an increase at 10 days and normalization of activity at 21 days. They also reported increased tissue content of prostaglandin at all three points of time (41).

Others have taken a different viewpoint regarding renal prostaglandin production and hypertension in the rat (42, 43). These workers observed renal vasoconstriction after administration of PGE$_2$ to rats and enhancement of the vasoconstrictor response to sympathetic nerve stimulation. These findings, coupled with the observation that renal 15-hydroxy prostaglandin dehydrogenase decreases in New Zealand genetic hypertensive rats, prompted Armstrong et al. to suggest that increased renal concentration of PGE$_2$ may cause this type of hypertension (43). Face-Asciak has recently reported that 15-hydroxy prostaglandin dehydrogenase is also decreased in the Japanese SHR (44).

The potential role of prostaglandin synthesis in the control of blood pressure is possibly elucidated by studies in normotensive and hypertensive animals in which prostaglandin production rates have been experimentally altered. The administration of indomethacin, a potent inhibitor of prostaglandin synthetase, acutely raises the blood pressure after intravenous use in normotensive rats (41) and rabbits (45, 46) and chronically elevates the blood pressure in normotensive rabbits (47). In Goldblatt rats and rabbits administration of indomethacin elevated blood pressure (10, 48, 49). In Japanese SHR ranging from 10–38 wk of age administration of indomethacin has been reported to increase blood pressure 40 mm Hg at 30 min, whereas there were no alterations of BP in 7–8 wk SHR or 23–32 wk WKy. Enhancement of prostaglandin synthesis by the acute administration of arachidonic acid substantially reduced blood pressure (10–60%) in normal rabbits (46) and in Okamoto SHR (50); furthermore this effect was blocked by indomethacin in both experiments. One must be cautious interpreting these results since the indomethacin and the arachidonic acid were given systemically and hence the specific role of the kidney in the systemic response of blood pressure is unknown. It should be noted that Tannenbaum et al. have found that acute infusions of arachidonic acid into the renal artery of normotensive dogs have no effect on systemic blood pressure despite significant acute changes of renal blood flow and sodium excretion (51). Simpson has reported enhanced vasodepressor activity of PGE$_2$ (but not of other vasodilators) in SHR when compared to three normotensive strains of rat (52).

We have been cautious with regard to the PGAs-like end-product of the synthetase reaction. Previous attempts by other investigators have not demonstrated direct enzymatic production of PGAs in the kidney (5, 30, 31). More refined techniques of identification of the PGAs-like spot (R$_t$ 0.66) are required to positively separate 15-keto-13, 14-dihydro PGAs hydroxy acids, PGFs, and PGA$_2$ (20). Furthermore, our use of glutathione in the incubation mixture for the synthetase activity.

reaction undoubtedly formed water soluble adducts with PGA and thereby diminished the extraction of PGA into the organic solvent used to spot the silica gel plates (53). Our inability to measure PGA in frozen rat medulla using radioimmunoassay suggests that the radio-labeled end-product with an Rf of 0.66 is not PGAs.

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