Renal Prostaglandin Excretion and Metabolism in Male and Female New Zealand Normotensive and Genetically Hypertensive Rats

PHILIP G. BAER, PH.D., AND LAUREN M. CAGEN, PH.D.

SUMMARY Reduced renal 15-hydroxyprostaglandin dehydrogenase (PGDH) activity has been proposed as a cause, subsequent to elevation of intrarenal prostaglandin (PG) E₂ levels, of the development or maintenance of high blood pressure (BP) in the New Zealand genetically hypertensive (NZGH) rat. To test this hypothesis, PGDH activity in homogenates of kidneys and lungs and in urine concentration and excretion of PGE₂ were determined in male and female NZGH and normotensive control (NZNR) rats. Lung PGDH activities of the four groups were similar. Renal PGDH activity was 50% lower for the male NZGH than for the male NZNR, but for the female rats no difference in renal PGDH activity was found between NZGH and NZNR. In addition, there was a large sex-related difference in renal PGDH activities, values for the female rats being only 5% to 10% of the values for males. Urine PGE₂ concentration and excretion were two to five times greater for the female rats than for the males, but did not differ between male NZGH and male NZNR. From these observations, it appears that neither renal PGDH activity nor urine PGE₂ levels is associated with hypertension in the New Zealand genetically hypertensive strain of rats. (Hypertension 3: 257-261, 1981)

KEY WORDS • genetic hypertension • prostaglandins • renal function

THE New Zealand genetically hypertensive (NZGH) strain of rat was developed, beginning in 1955, from a closed colony of New Zealand normotensive (NZNR) Wistar rats. Although the causal mechanism underlying the development of high blood pressure (BP) in this strain remains undefined, it has been proposed that altered prostaglandin (PG) metabolism in the kidney may be a contributing factor. The basis for this proposal rests on several observations. Armstrong et al. found that the activity of 15-hydroxyprostaglandin dehydrogenase (PGDH) in kidneys from male NZGH is only about 30% of that in kidneys from male NZNR, but that renal prostaglandin (PG) synthetase activities were not different. PGDH is a major prostaglandin-catabolizing enzyme in rat kidney, oxidizing PGE₂ and PGF₂α to 15-keto metabolites, and its activity could be a determinant of intrarenal levels of these two prostaglandins. PGE₂ has been shown to vasoconstrict the rat kidney and to potentiate renal vascular responses to the vasoconstrictor effects of nerve stimulation and infused norepinephrine. From these observations, it was proposed that increased PGE₂ levels in the kidney, resulting from lower PGDH activity, might contribute to the development or maintenance of hypertension in the NZGH, through direct or indirect renal vasoconstrictor effects of PGE₂.

We recently observed, in normal Wistar rats, that PGDH activity is approximately 40 times greater in the kidneys of males than in the kidneys of females; this difference between male and female normotensive rats is far greater than the differences reported between genetically hypertensive and normotensive male rats. In the present study, we have compared renal prostaglandin metabolizing activity, and urinary prostaglandin excretion, in male and female rats of the New Zealand genetically normotensive and hypertensive strains. In addition to confirming that PGDH activity is lower in the kidneys of male NZGH than in the kidneys of male NZNR, we found that renal PGDH activity of female NZGH and NZNR is much lower than that of the males of the two strains. In addition, we found no difference between the PGDH activities of female NZGH and female NZNR kidneys.

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Methods and Materials

In this study we used 18-week-old male and female New Zealand normotensive (NZNR) and genetically hypertensive (NZGH) rats from the colony maintained at this institution. Foundation breeders were obtained in 1976 from the Wellcome Medical Research Institute. Prior to the beginning of our study, rats were grouped three or four to a cage, in a room maintained at 70°-72°F, relative humidity 50%, on a 12-hour light cycle. Tap water and standard rat chow (Purina 5001) were available ad libitum. There were five rats in each of the four groups studied.

For 24-hour urine collection studies, rats were placed in individual metabolic cages; during a 3-day adaptation period, systolic BP was measured on two occasions by tail plethysmography after warming the rats at 37°C for 10 minutes. When systolic BP was measured on 6 consecutive days in a randomly selected group of five female NZGH, the coefficient of variation for this method was 7.6%. Following the adaptation period, three consecutive 24-hour urine collections were taken; urine containers were kept on dry ice so that the urine was frozen immediately after the animals voided. After completion of the urine collection, the rats were killed by decapitation, and the lungs and both kidneys were removed and frozen on dry ice and acetone within 1 minute.

PGDH Activity in Kidneys and Lungs

Kidneys or lungs were thawed into 4 volumes of 0.01 M Tris HCl buffer, pH 7.8, containing 0.1 mM dithiothreitol. Tissue was sliced into small fragments, disrupted with a Brinkman Polytron homogenizer (30 sec at top speed), and centrifuged at 4250 X g for 15 minutes. Nicotinamide adenine dinucleotide (NAD+), (2 μmoles), (1-14C)-PGDH (0.01 μCi, 200 pmoles), and Tris-HCl-dithiothreitol buffer were incubated with lung or kidney supernatant in a total volume of 1 ml for 15 minutes at 37°C. The reaction was then terminated by the addition of 10 μl of formic acid, and the acidified solutions were immediately placed on ice until extracted three times with 1 ml of ethyl acetate.

No nonenzymic decomposition of PGDH was observed during incubation with boiled enzyme. To control for the slight (1% to 2%) dehydration of PGDH that occurs during preparation of samples for chromatography, in some tubes in each series 10 μl of formic acid was added to the reaction mixture prior to the addition of (1-14C)-PGDH, and the solution was extracted without incubation. After removal of solvent under a stream of nitrogen, residue in the organic phase was redisolved in 50 μl of methanol and applied to a silica gel G/h thin layer plate (0.1 mm; Brinkman) which was developed in ethyl acetate/isooctane/acetic acid/water (110/55/20/100; upper phase). Radioactive zones were located by radiochromatogram scanning (Packard Model 7201). Zones corresponding in mobility to PGDH (Rf: 0.57), 13,14-dihydro 15-keto PGDH (Rf: 0.61), or PGA2 (Rf: 0.65) standards were scraped from the plate and suspended in 10 ml Scintiverse cocktail (Fisher), and radioactivity was determined by liquid scintillation counting (Searle Delta 300).

The extent of metabolism was determined from the equation: pmole PGDH metabolized = (% of radioactivity in metabolite zones - % of radioactivity in metabolite zones in control incubations) X pmole PGDH added to the reaction mixture. All values reported are the mean of duplicate determinations, and are expressed as pmole PGDH oxidized in 15 min/mg protein; protein concentration was determined by the method of Lowry et al.11

Radioimmunoassay for Urinary PGDH

Urine content of immunoreactive PGDH was determined by radioimmunoassay according to the method of Dray et al. after extraction and partial purification by silicic acid chromatography. Aliquots (2 ml) of the urine sample were diluted to a final volume of 10 ml with water, acidified to pH 3.0 with formic acid after addition of 4H-PGE2 tracer, and extracted twice with cyclohexane/ethyl acetate (1/1). The organic phase was removed and evaporated, and the residue was redissolved for chromatography on 0.5 g silicic acid (AR grade, 100 mesh; Mallinckrodt, Paris, Kentucky) columns equilibrated with benzene/ethyl acetate (3/2). After development of the columns with 5.5 ml of benzene/ethyl acetate (3/2) to elute PGAD and PGB, with 12 ml of benzene/ethyl acetate/methanol (30/20/1) to elute PGE, and with 5 ml of benzene/ethyl acetate/methanol (3/2/1) to elute PGB, the fractions of interest were dried under a stream of nitrogen and redissolved in assay buffer (6 ml) the day of assay.

For the assay procedure, standards of PGDH (0.95-500 pg) or sample (100 μl aliquots assayed in triplicate) were incubated with antibody (Pasteur Institute, Paris) for 2 hours at 4°C. Bound and free antigen were separated with charcoal-dextran, and the concentration of antibody-bound antigen was determined by scintillation counting of the supernatant. Recovery through the extraction and purification procedures was determined for each sample from tracer 4H-PGE2 recovery, and assay estimates were individually corrected for losses. All values reported in this study were obtained from a single assay. Intra-assay variability was assessed by eight replicate assays of pooled rat urine; the coefficient of variation for those determinations was 8.2%.

Analysis of Data

Values in the text, figures, and tables are expressed as mean ± the standard error of the mean (SEM). Differences between group mean values were analyzed by Student's t test for unpaired observations; a p value of less than 0.05 was taken to indicate statistical significance.
Results

As shown in table 1, male rats weighed more than female rats of the same strain, and male and female NZNR were heavier than their NZGH counterparts. Kidney weights, normalized for differences in body weights, were not different for the male NZGH and NZNR; for the females, the relative kidney weight of the NZGH was slightly, but significantly, greater than that of the NZNR. Systolic BP of the NZGH was 40–50 mm Hg higher than that of the NZNR; within the two strains, the BP of the male and female rats did not differ significantly.

The 15-hydroxyprostaglandin dehydrogenase (PGDH) activity of the kidneys and lungs from the four groups is shown in figure 1. For the lungs (upper panel) no difference was observed either between sexes or between strains within the same sex. In contrast to the consistency of PGDH activity in the lungs from the four groups, PGDH activity in kidneys of the different groups was found to differ greatly. For the male rats, PGDH activity in the kidneys of the NZNR was approximately twice that of the NZGH. This strain-related difference was seen only for the male rats, however; for the females, PGDH activity in the kidneys was similar for the NZGH and NZNR. In addition to the strain-related difference between male rats, there was an even larger sex-related difference within both the normotensive and hypertensive strains. Female rat kidney PGDH activity was only 5.3% of that of the males for the NZNR, and 10% for the NZGH.

Twenty-four-hour urine volumes and urine concentrations and excretion rates of PGE₂ are shown in table 2. Because body weights varied among the four groups (see table 1), the urine volumes and prostaglandin excretion rates are expressed both as absolute values and as per 100 g of body weight. Absolute urine volumes did not differ significantly among the four groups; however, when factored for body weight differences, urine volumes of the NZGH rats were significantly greater than those of the NZNR of the same sex, and the urine volumes of the female rats were greater than those of the male rats of the same strain. For the male rats, urine PGE₂ concentration and excretion, whether absolute or factored for body weight, were similar in the NZGH and NZNR. Urine PGE₂ concentration for the female rats was significantly greater, by two- to fivefold, than for the male rats of the same strain; in addition, there was

![Figure 1. Activity of 15-hydroxyprostaglandin dehydrogenase in whole organ homogenates of kidneys and lungs from male and female New Zealand genetically hypertensive and normotensive rats, n = 5 in each group. Vertical lines indicate standard error of the mean; * indicates p < 0.001.](http://hyper.ahajournals.org/)

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Body Wt (g)</th>
<th>Kidney Wt (g)</th>
<th>KW/100 g BW</th>
<th>SBP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZNR—M</td>
<td>363 ± 13</td>
<td>1.36 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>119 ± 2</td>
</tr>
<tr>
<td>p &lt;</td>
<td>0.001</td>
<td>0.01</td>
<td>ns</td>
<td>0.001</td>
</tr>
<tr>
<td>NZGH—M</td>
<td>282 ± 9</td>
<td>1.13 ± 0.04</td>
<td>0.40 ± 0.01</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>NZNR—F</td>
<td>251 ± 13</td>
<td>0.89 ± 0.04</td>
<td>0.36 ± 0.01</td>
<td>126 ± 3</td>
</tr>
<tr>
<td>p &lt;</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>NZGH—F</td>
<td>177 ± 6</td>
<td>0.75 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>169 ± 3</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM; n = 5 in all groups, p values refer to comparisons between NZGH and NZNR of the same sex. M = male; F = female, NZNR = normotensive control New Zealand rat; NZGH = genetically hypertensive New Zealand rat; ns = not significant.
nearly a threefold difference in urine PGE₂ concentration between the two groups of female rats, being significantly higher in the NZNR. Urine PGE₂ excretion by the female rats significantly exceeded that of the male rats of the same strain; this difference was seen whether PGE₂ excretion was compared as absolute or body-weight-factored values. As seen for the urine PGE₂ concentration, urine PGE₂ excretion by the female NZNR significantly exceeded that of the female NZGH.

Discussion

Although the mechanism underlying the development and maintenance of high BP has not been unequivocally defined for any of the various strains of genetically hypertensive rats, several studies implicate altered renal function as a contributory factor. For the New Zealand genetically hypertensive strain of rats, Armstrong et al. set forth the hypothesis that altered intrarenal prostaglandin catabolism causes hypertension or is involved in its maintenance. That proposal was based on the following observations. PGE₂, a major prostaglandin of renal origin, constricts the rat renal vasculature and potentiates the vascular response of the rat kidney to other vasoconstrictor stimuli, including renal nerve stimulation and infusion of norepinephrine. Although PGDH activity is lower in the kidneys from male NZGH rats than in those from male NZNR, there is no difference in the rate of production of prostaglandins from exogenous arachidonic acid by homogenates of the two strains, suggesting that prostaglandin synthetase activity is not different in the NZGH and NZNR kidneys. Because PGDH is a major prostaglandin-catabolizing enzyme in rat kidney, converting PGE₂ and PGF₂α to 15-keto metabolites, it was assumed that the combination of decreased PGDH activity and equal prostaglandin synthetase activity would result in elevated intrarenal PGE₂ levels, and that the direct and/or indirect vasoconstrictor effects of PGE₂ in the rat kidney would contribute to the development or maintenance of hypertension in the NZGH.

The results of the present study bear on two assumptions fundamental to this hypothesis: first, that intrarenal PGE₂ levels in the intact animal vary reciprocally with renal PGDH activity measured in vitro; and, second, that high levels of PGE₂ in the kidney, or low levels of PGDH activity, are associated with hypertension. The term "renal prostaglandin level" is unavoidably ambiguous. Prostaglandins are not stored in the kidney but, rather, are produced on demand at or near their site of action and rapidly excreted or metabolized. The renal prostaglandin content, therefore, may not reflect accurately the overall state of activity of the renal prostaglandin system. While we are aware that a relationship has not been established between urine PGE₂ concentration or excretion and renal PGE₂ levels, several observations are consistent with the assumption that, if renal PGDH activity is a determinant of intrarenal PGE₂ levels, this will be reflected in the urine PGE₂ concentration and/or excretion rate. The PGE₂ appearing intact in the urine is considered to be of renal origin, entering the tubular fluid of the loop of Henle. Oxidation to 15-keto metabolites may occur during passage of the tubular fluid through the more distal portions of the nephron; in the rat kidney, PGDH activity appears predominantly in the thick ascending limb of the loop of Henle.

Our findings do not support the hypothesis that renal PGDH activity is a factor in the determination of BP levels in New Zealand genetically hypertensive rats. This statement is based on the following observations. First, although we found, as have others, that for males renal PGDH activity is lower in NZGH rats than in the NZNR, there is no corresponding difference in the renal PGDH activity in the kidneys of female NZGH and NZNR; there is no difference in the degree to which hypertension develops in the male and female NZGH, and no correlation between renal PGDH activity and systolic BP for either sex. Second, PGDH activities of both NZGH and NZNR female rat kidneys, while not different from each other, are markedly lower than the activities of kidneys of males of either strain. Although this difference between males and females is greater than...
any difference found between male rats of hypertensive and normotensive strains, the BPs of male and female rats of the same strain do not differ. Third, the difference in PGDH activity in the kidneys of male NZGH and NZNR does not result in a different urine PGE₂ concentration or rate of excretion. Further, the supposition that intrarenal PGE₂ levels can be assumed from in vitro PGDH measurements may be invalidated by the finding that, although female rats did excrete more PGE₂ in the urine than males, the excretion by the female NZNR was three times that by the female NZGH and yet there was no difference between the renal PGDH activities of the kidneys of these two groups of females. Finally, based on the assumption that urine PGE₂ concentration or excretion reflects intrarenal PGE₂ levels, we found no support for the proposal that elevated renal PGE₂ levels are pro-hypertensive in the rat, since both the urine concentration and excretion of PGE₂ were much higher in the female normotensive rats than in either of the groups of hypertensive rats.

The difference between PGDH activities of kidneys of male and female rats is clearly not characteristic of all tissues. In the lungs, which have been shown to remove most, if not all, PGE₂ from the blood during passage through the pulmonary circulation, we observed no difference in PGDH activity between the sexes; in addition, we found no difference between NZGH and NZNR of the same sex. This latter observation suggests that the prostaglandin-metabolizing activity of various organs of rats may vary with the strain studied; Tai et al. recently showed in male rats that PGDH activity of the lungs of spontaneously hypertensive rats (SHR) is twice that of normotensive (WKY) control rat lungs. In that same study it was found, as reported by others, that PGDH activity in the kidneys of male SHR is 30% to 50% lower than that in the kidneys of male WKY.

Conclusions

The purpose of this study was to test several assumptions of the hypothesis that the development and/or maintenance of high BP in the New Zealand genetically hypertensive rat is due to reduced activity of 15-hydroxyprostaglandin dehydrogenase in the kidney. Our observations do not support these assumptions. First, the difference in renal PGDH activity between NZGH and NZNR was demonstrable between male rats, but not between female rats. Second, low renal PGDH activity is not necessarily associated with hypertension, since the differences between male and female NZNR were much greater than the differences between male NZGH and male NZNR. Third, renal homogenate PGDH activities and renal PGE₂ levels did not appear to be consistently related, since female NZNR and NZGH had similar PGDH values but greatly dissimilar urine PGE₂ concentrations, and male NZGH and NZNR had similar urine PGE₂ levels but dissimilar renal PGDH values. Finally, elevated intrarenal PGE₂ levels were not found to be associated with hypertension, since the highest urine PGE₂ concentrations were found in the normotensive females.

Acknowledgments

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References

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