Altered vitamin D metabolism in the kidney of the spontaneously hypertensive rat

Hiroyuki KAWASHIMA*
Nephrology Section, Medical and Research Services, Veterans Administration Wadsworth Medical Center and Department of Medicine, University of California School of Medicine, Los Angeles, CA 90073, U.S.A.

A decrease in plasma Ca\(^{2+}\) and increases in plasma immunoreactive parathyroid hormone (PTH) have been reported in spontaneously hypertensive (SH) rats as compared with normotensive Wistar–Kyoto (WKy) rats. These changes should lead to a higher plasma 1,25(OH)\(_2\)D (1,25-dihydroxycholecalciferol/1,25-dihydroxyergocalciferol) concentration in SH rats if the kidney responds appropriately. Plasma 1,25(OH)\(_2\)D, however, has been reported to be normal in SH rats, suggesting possible impairments of vitamin D metabolism in this animal model of hypertension. To test this possibility, we studied the effect of PTH on renal production of 1,25(OH)\(_2\)D in SH rats before (4 weeks of age) and after (12 weeks of age) the onset of hypertension. Basal serum levels of 1,25(OH)\(_2\)D were normal in SH rats at both ages. At 4 weeks of age, the rise in serum 1,25(OH)\(_2\)D after PTH injection (50 units subcutaneously every 2 h; four times) was also normal in SH rats. By contrast, at 12 weeks of age, the rise in serum 1,25(OH)\(_2\)D was approximately one-half of that in WKy rats, despite the similar rises in serum Ca\(^{2+}\) levels in both groups by PTH injection. The attenuated rise in serum 1,25(OH)\(_2\)D in SH rats was consistent with the impaired response of renal 1-hydroxylase (25-hydroxycholecalciferol 1α-hydroxylase) activity to PTH. Basal 1,25(OH)\(_2\)D production by the kidney in SH rat was higher than that in WKy rats both at 4 and 12 weeks of age. These data suggest that, in SH rats: (1) serum 1,25(OH)\(_2\)D is inappropriate low in relation to the elevated PTH and this may be due, at least in part, to the impaired responsiveness to PTH of renal 1-hydroxylase and to the enhanced metabolism of 1,25(OH)\(_2\)D, and (2) elevated PTH or other agents may stimulate the 1-hydroxylase in the kidney even before the onset of hypertension.

INTRODUCTION

Accumulating evidence suggests that calcium homoeostasis has a significant role in the development and maintenance of hypertension. Dietary calcium intake is lower in patients with hypertension than in normotensive individuals (Belizan & Villar, 1980). In epidemiological studies, low dietary calcium has been associated with hypertension (Neri et al., 1972; Masironi et al., 1976). Low calcium intake increases blood pressure in WKy rats (McCarron, 1982a), which are controls for SH rats developed from this strain. High calcium intake lowers blood pressure in SH rats (Ayachi, 1979; McCarron et al., 1981; Chen et al., 1982; Schneiderman et al., 1984) as well as in WKy rats (Ayachi, 1979; Schneiderman et al., 1984).

Total serum calcium is similar in patients with essential hypertension and in normotensive controls, but serum ionized calcium (Ca\(^{2+}\)) is decreased in hypertensive patients (McCarron, 1982b). This is also true in SH rats as compared with WKy rats; under comparable feeding conditions, serum Ca\(^{2+}\) is lower in SH rats than in WKy rats (McCarron et al., 1981; Wright et al., 1980; Wright & Rankin, 1982). Serum immunoreactive PTH is elevated in patients with essential hypertension (McCarron et al., 1980) and in SH rats (McCarron et al., 1981). Intestinal Ca\(^{2+}\) absorption is decreased (Schedl et al., 1984), unchanged (Stern et al., 1984) or less responsive to exogenous 1,25(OH)\(_2\)D \(_3\) (Torasorn & Wright, 1981) in SH rats. Plasma 25(OH)D\(_2\) is lower in patients with hypertension treated with hydrochlorothiazide (Kokot et al., 1981) and higher in SH rats than in WKy rats (Schedl et al., 1984), whereas plasma 1,25(OH)\(_2\)D\(_3\) is reported to be normal in SH rats at 12 weeks of age (Schedl et al., 1984; Stern et al., 1984).

These findings suggest abnormalities in calcium homoeostasis in this animal model of hypertension, particularly in vitamin D metabolism, since increased plasma PTH levels seem to fail to raise 1,25(OH)\(_2\)D above normal values. To detect possible defects in the animal model of essential hypertension, we studied the responsiveness to PTH of renal 1-hydroxylase and serum concentration of 1,25(OH)\(_2\)D in SH and WKy rats.

EXPERIMENTAL

Animals

Male SH and WKy rats were purchased from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) and fed a Purina rat chow ad libitum. Rats, at 4 and 12 weeks of age, were used in this study. Animals, at each age, were divided into two groups. A group of rats was given a subcutaneous injection of PTH (Beckman...
Instrument Company, Palo Alto, CA, U.S.A.) every 2 h (four times). The control group received saline (0.9% NaCl) as a vehicle. At 2 h after the last injection, rats were exsanguinated by aortic puncture and sera were collected for the determination of calcium and 1,25(OH)₂D₃. Kidneys were excised for the assay of the 1,25(OH)₂D₃ production. Serum calcium concentration was measured by the orthocresolphthalein complexone method (Connerty & Briggs, 1966). Blood pressure was monitored with an electrophysgmomanometer (Narco Biosystem, Houston, TX, U.S.A.) on the day before the experiment.

To examine renal function, creatinine clearance was measured in another set of experiments. Animals were anaesthetized with sodium pentobarbitonal (40 mg/kg, intraperitoneally) and were infused via a femoral arterial catheter at a rate of 1 ml/h with a solution composed of 5 mM-CaCl₂, 5 mM-MgCl₂, 20 mM-NaCl, 2.5 mM-KCl and 4% (w/v) glucose, starting at 5 h. Blood samples were obtained by a catheter inserted into the femoral vein. Bladder was also catheterized and urine was collected from 6 to 8 h. Endogenous creatinine concentrations in urine and serum were determined by the method of Bonsnes & Tauskky (1945).

**Serum 1,25(OH)₂D₃ assay**

The serum concentration of 1,25(OH)₂D₃ was determined by the method of Reinhardt et al. (1984), with bovine thymic receptor for 1,25(OH)₂D₃ (see below).

**Production of 1,25(OH)₂D₃ by the kidney**

Mitochondria were prepared from the kidney, and incubations were performed by the method of Vieth & Fraser (1979). The kidneys were placed in an ice-cold homogenization medium (250 mM-sucrose/10 mM-Hepes/10 mM-KCl, adjusted with NaOH to pH 7.42 at room temperature) and cortical tissues were obtained after removing capsules, papilla, ureter and medulla. The tissues were homogenized in 10 vol. (w/v) of ice-cold homogenization medium with a motor-driven Potter-Elvehjem homogenizer. The homogenate was centrifuged at 4000 g and 4 °C for 40 s, and the supernatant was centrifuged at 9000 g and 4 °C for 20 min. The pellet was suspended in 10–15 vol. of ice-cold incubation medium (125 mM-KCl/20 mM-Hepes/10 mM-L-malic acid/2 mM-MgSO₄/1 mM-dithiothreitol/0.25 mM-EDTA), adjusted to pH 7.42 at room temperature; the final concentration of mitochondrial protein was 2.5–5 mg/ml. Each incubation sample consisted of a 1.0 ml portion of mitochondrial suspension. Samples, in 25 ml Erlenmeyer flasks, were placed in a Dubnoff incubator at 25 °C, shaking at 100 cycles/min. At 3 min, 5 μg of 25(OH)D₃ (kindly provided by the Upjohn Co., Kalamazoo, MI, U.S.A.) in 20 μl of ethanol were added. After substrate addition, the flask was gassed for 1 min with a direct flow of O₂/CO₂ (19:1) at a rate of 0.5 litre/min. Immediately after gassing, the flask was sealed with a rubber stopper, incubated at 25 °C for 15 min and the reaction was stopped by the addition of 3.75 ml of methanol/chloroform (9:1:1, by vol.), and then subjected to Sephadex LH-20 column chromatography. The column was eluted with the same solvent at a rate of 0.6 ml/min, and fractions (3 ml each) were collected. Fractions (nos. 63–74) containing 1,25(OH)₂D₃ were pooled, dried under an N₂ gas stream and resolved in n-hexane and propan-2-ol (9:1, v/v), and subjected to h.p.l.c. (μPorasil; column size 0.39 cm × 30 cm). The column was eluted with the same solvent and 1,25(OH)₂D₃ fractions were collected. This 1,25(OH)₂D₃ fraction was applied to another h.p.l.c. system (μBondapak C₁₈) and eluted with water/methanol (7:3, v/v). The 1,25(OH)₂D₃ fractions were collected and extracted again by the method of Bligh & Dyer (1959). The extracted 1,25(OH)₂D₃ was dried under an N₂ stream and stored at −70 °C until the time of assay. 1,25(OH)₂D₃ was determined by the method of Reinhardt et al. (1984), with bovine thymic receptor. In short, standard solutions of 1,25(OH)₂D₃ (generously given by Dr. Uskokovic of Hoffman-La Roche, Nutley, NJ, U.S.A.) (1–64, and 800 pg, for non-specific binding determination) and samples (in 30 μl of ethanol) were added to 12 mm × 75 mm glass tubes on ice. Freeze-dried receptor was solubilized and diluted in a buffer containing 50 mM-Tris/HCl, 500 mM-KCl, 5 mM-dithiothreitol, 10 mM-Na₂MoO₄ and 1.5 mM-EDTA, pH 7.5, and then 450 μl of receptor solution (approx. 0.7 mg of protein/tube) was added to the standards and samples on ice, followed by vortex-mixing. The samples and standards were incubated in a 25 °C water bath for 45 min with gentle shaking. At the end of 45 min the tubes were transferred to an ice bath and allowed to cool for 5 min, and then each tube received 5000 c.p.m. of [26,27-methyl-³H]1,25(OH)₂D₃ (160 Ci/mmol) (Amersham, Arlington Heights, IL, U.S.A.) in 25 μl of ethanol. The tubes were vortex-mixed and the incubation was continued for 15 min in a 25 °C water bath with shaking. The tubes were allowed to cool for 5 min in an ice bath, and 200 μl of dextran-coated charcoal suspension were added to each tube, followed by vortex-mixing. The tube contents were vortex-mixed again after 10 min and, after 2 min of charcoal treatment, bound and free hormones were separated by centrifugation at 2000 g for 10 min. The supernatant containing bound hormones was decanted into a scintillation vial and radioactivity was determined in a liquid-scintillation counter (Beckman; model 8100) (counting efficiency approx. 40%).

**Protein determination**

The mitochondrial protein concentration was measured by the method of Lowry et al. (1951), with bovine serum albumin as standard.

**Calculations**

The 1,25(OH)₂D₃ value (pg/tube) was calculated by using a logit-log plot of the data. The 1,25(OH)₂D₃ concentration of serum (pg/ml) or 1,25(OH)₂D₃ production by tissues (fmol/mg of protein) were obtained after correcting for recovery (approx. 50%) and sample volume.

**Statistical analysis**

Results are given as ± S.E.M. and statistical analysis was performed by Student's t test.
Table 1. Body weights and blood pressures of WKy and SH rats

Blood pressure was measured the day before the experiment by using an electrosphygmomanometer, and body weight was measured before the start of the experiment. Abbreviation used: n.s., not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (weeks)</th>
<th>No. of animals</th>
<th>Blood pressure (mean ± S.E.M.) (mm Hg)</th>
<th>Body weight (g) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKy</td>
<td>4</td>
<td>8</td>
<td>83 ± 5</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>SH</td>
<td>4</td>
<td>8</td>
<td>92 ± 7</td>
<td>47 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Significance (WKy versus SH)...</strong></td>
<td></td>
</tr>
<tr>
<td>WKy</td>
<td>12</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>12</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significance (WKy versus SH)...**

Table 2. Effect of PTH on the serum concentrations of calcium and 1,25(OH)₂D

Rats received subcutaneous injections of either PTH or vehicle at 0, 2, 4 and 6 h. Blood samples were collected at 8 h. Abbreviation used: n.s., not significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (weeks)</th>
<th>No. of animals</th>
<th>1,25(OH)₂D (pg/ml) (mean ± S.E.M.)</th>
<th>Calcium (mg/100 ml) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>PTH</td>
</tr>
<tr>
<td>WKy</td>
<td>4</td>
<td>4</td>
<td>135.0 ± 8.3</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>SH</td>
<td>4</td>
<td>4</td>
<td>123.7 ± 18.4</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Significance (WKy versus SH)...</strong></td>
<td>n.s.</td>
</tr>
<tr>
<td>WKy</td>
<td>12</td>
<td>4</td>
<td>50.1 ± 8.3</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>SH</td>
<td>12</td>
<td>4</td>
<td>37.4 ± 4.2</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Significance (WKy versus SH)...</strong></td>
<td>n.s.</td>
</tr>
</tbody>
</table>

RESULTS

Table 1 lists the body weights and blood pressures of WKy and SH rats used in the present study. At 12 weeks of age the systolic blood pressure of SH rats was significantly elevated. At 4 weeks of age, on the other hand, the blood pressure of SH rats remained normal.

Serum total calcium concentrations of SH rats were in the same range as those of WKy rats at either age (Table 2). There was no significant difference in serum 1,25(OH)₂D between SH and WKy rats at either age, although the concentration in SH rats tended to be lower than that in age-matched WKy rats (Table 2). When rats were treated with PTH, however, a marked difference in serum 1,25(OH)₂D of SH rats with age was observed. At 4 weeks of age, serum 1,25(OH)₂D increased 4-fold in SH rats, which is similar to the value obtained for WKy rats. By contrast, at 12 weeks of age, the increase in serum 1,25(OH)₂D in SH rats was approximately one-half that in WKy rats (Table 2). The serum 25(OH)D levels were in the same range for both SH and WKy rats (28.3 ± 0.7 ng/ml and 26.6 ± 0.7 ng/ml respectively). Rises in serum calcium concentration in SH and WKy rats at either age did not differ (Table 2).

Fig. 1 depicts the effect of PTH on the renal production of 1,25(OH)₂D in SH and WKy rats. The basal 1,25(OH)₂D productions by the renal mitochondria from SH rats were significantly higher than those from WKy rats both at 4 weeks of age (143.4 ± 17.6 vs. 48.0 ± 8.3 fmoi/mg protein) and at 12 weeks of age (90.6 ± 16.4 vs. 45.8 ± 7.2 fmoi/mg protein). Upon PTH injection, renal 1,25(OH)₂D production of SH rats at 4 weeks of age increased to 713.7 ± 23.4 fmoi/mg of protein, a value that is not significantly different from that for WKy rats, namely 976.8 ± 120.2 fmoi/mg of protein (Fig. 1). On the basis of these data and the fact
Table 3. Creatinine clearance in SH and WKy rats at 12 weeks of age

Rats were treated as described in Table 2 and anaesthetized with sodium pentobarbital. Rats were infused at a rate of 1 ml/h with a solution composed of 5 mm-CaCl₂, 5 mm-MgCl₂, 20 mm-NaCl, 2.5 mm-KCl and 4% (w/v) glucose, starting at 5 h. Urine was collected from 6 to 8 h via a catheter inserted into the bladder. Serum samples were obtained via a catheter inserted through the femoral vein. Abbreviation used: n.s., not significant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>Creatinine clearance (ml/h per 100 g body wt.) (mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKy</td>
<td>4</td>
<td>Control 0.68 ± 0.8, PTH 0.65 ± 0.7</td>
</tr>
<tr>
<td>SH</td>
<td>4</td>
<td>Control 0.71 ± 0.8, PTH 0.63 ± 0.5</td>
</tr>
<tr>
<td>Significance (WKy versus SH)...</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

that the dosage level of PTH used in the present study is known to stimulate maximally the enzyme activity, thereby leading to a saturation of the enzyme activity, it is reasonable to assume that the responsiveness of the enzyme to exogenous PTH in SH rats at this age remains intact. By contrast, renal 1,25(OH)₂D production after PTH injection in SH rats at 12 weeks of age increased to only 202.3 ± 23.9 fmol/mg of protein, which is much less than in WKy rats, namely 738.8 ± 97.3 fmol/mg of protein (Fig. 1).

As Table 3 shows, no difference in the creatinine clearance was observed between SH and WKy rats at 12 weeks of age.

DISCUSSION

The present study supports the previous observations by others (Shedl et al., 1984; Stern et al., 1984) that serum 1,25(OH)₂D in SH rats is in the normal range. Furthermore, our data demonstrate that rises in serum 1,25(OH)₂D in response to PTH injection are attenuated at 12 weeks of age but not at 4 weeks of age.

The reduced response of serum 1,25(OH)₂D concentration to PTH injection may be due to either reduced stimulation of 1-hydroxylase in the kidney or enhanced metabolism of 1,25(OH)₂D. To test these possibilities, we measured 1-hydroxylase activity in the kidney. The enzyme activity in renal mitochondria from SH rats at 4 weeks of age responded properly to PTH (Fig. 1). By contrast, from SH rats at 12 weeks of age showed a markedly reduced response, suggesting that the decreased serum 1,25(OH)₂D in response to PTH injection may be mainly due to the impaired response to PTH of 1-hydroxylase (Fig. 1).

Although normal renal function has been reported for SH rats as compared with age-matched WKy rats, it is possible that the observed reduction in response to PTH of 1-hydroxylase in SH rats at 12 weeks in the present study may have been a result of reduced renal function. It turned out not to be the case, however, since creatinine clearance in SH rats at this age did not differ from that in WKy rats (Table 3). Thus the attenuated response to PTH of 1-hydroxylase seems to be attributable to the decreased responsiveness of the enzyme activity itself. Two possible mechanisms may account for the attenuated responsiveness of the enzyme activity to PTH. One is that, with existing high levels of PTH in the SH rats, the renal 1-hydroxylase is incapable of further substantial increase in activity. This may be partly true, since basal 1,25(OH)₂D production by the kidney was higher in the SH rats as compared with that in WKy rats (Fig. 1), a finding that is consistent with the reported hyperparathyroidism. However, since PTH has been known to inhibit phosphate reabsorption in the renal proximal convoluted tubules where 60–70% of filtered load of phosphate is reabsorbed, and since the phosphaturic effect of PTH was greater in SH rats than in WKy rats (670 ± 85 mg/100 ml of urine as against 432 ± 69 mg/100 ml of urine) in the present study, it is unlikely that the inhibitory effect of PTH on the reabsorption of phosphate in this nephron segment is impaired in SH rats. It is well established that PTH activates the adenylate cyclase system through binding to its receptor in the proximal convoluted tubules, which, in turn, leads to the inhibition of phosphate reabsorption as well as to the stimulation of 1-hydroxylase. Taken together, the attenuated response of 1-hydroxylase to PTH in SH rats observed in the present study could not be explained by a simple down-regulation of receptor due to the existence of higher PTH levels. Another possibility is that the SH rat may have a general defect in the transport of cell calcium that itself could result in secondary impairment of the control of the 1-hydroxylase. Decreases in urinary calcium excretion after PTH injection were, however, in the same range in SH and WKy rats (5.1 ± 0.6 and 3.8 ± 0.5 mg/ml respectively), thus suggesting that cellular calcium transport in the kidney of SH rat remains intact, although the possibility that the intracellular calcium concentration remains higher in SH rats is not ruled out.

No data are available on serum PTH in SH rats at 4 weeks of age. However, as shown in Fig. 1, basal 1,25(OH)₂D production by the kidney from SH rats was higher than that in WKy rats. These data suggest hyperparathyroidism or the presence of agents that stimulate the renal 1-hydroxylase activity in SH rats even at 4 weeks of age, when blood pressure is normal. Similar serum 1,25(OH)₂D levels in SH and WKy rats, notwithstanding the higher basal 1,25(OH)₂D production by the kidney in SH rats (Fig. 1), suggest possible enhancement of metabolism and/or excretion of 1,25(OH)₂D.

These data suggest that abnormality of calcium metabolism and, in particular, vitamin D metabolism, may contribute to the development of hypertension. Elucidation of (1) the mechanism underlying the effect of age on the impaired response to PTH of renal 1-hydroxylase and (2) the cause of higher basal enzyme activity even before the onset of hypertension, as well as the cause of enhanced metabolism of 1,25(OH)₂D, may provide further insight into pathophysiology of hypertension.

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Vitamin D metabolism in the spontaneously hypertensive rat

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