The Effects of Parathyroidectomy on the Development of Nephrocalcinosis in Rats Fed Phosphate-Supplemented and Unsupplemented Diets Containing Alpha Protein

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The effects of parathyroidectomy (PTX) on the development of nephrocalcinosis in rats fed a diet containing alpha protein were investigated for the purpose of determining whether the nephrocalcinosis was phosphate-induced. PTX completely prevented the occurrence of nephrocalcinosis in rats fed a phosphate-supplemented commercial laboratory diet for 4 weeks. However, PTX did not completely prevent the occurrence of nephrocalcinosis in rats fed a phosphate-supplemented alpha protein diet. Several calciferous deposits were found in the inner medulla. The same was also found in rats that underwent sham operations and PTX rats fed the basal alpha protein diet. Total renal calcium and phosphorous levels in these three groups were also similar and were about twice as great as those in corresponding groups fed phosphate-supplemented and unsupplemented commercial laboratory diets. Therefore, we conclude that the nephrocalcinosis in rats fed a basal alpha protein diet is not induced by PTH or excess phosphate, but is induced by some other factor associated with the diet. (Am J Pathol 1983, 113: 107-111)

THERE IS considerable evidence that female rats fed diets containing alkali-treated soy protein develop nephrocalcinosis. It has been suggested that the inorganic phosphate within the diet might be, at least in part, responsible for the nephrocalcinosis. When, however, an excess of phosphate is added to such a soy protein diet, a different form of renal calcification is found, one that is more typical of nephrocalcinosis induced by dietary phosphate, which has been described previously. Therefore, it seems that some of the mechanisms involved in the development of nephrocalcinosis in rats fed a diet containing alkali-treated soy protein are different from those involved in nephrocalcinosis in rats given excess dietary phosphate. This still does not preclude the possibility that the phosphate content of a basal alkali-treated soy protein diet is, in some way, involved in the calcification process.

Evidence suggests that phosphate-induced nephrocalcinosis is mediated by parathyroid hormone (PTH). In particular, it has been shown that parathyroidectomy (PTX) prevents nephrocalcinosis in male rats treated with phosphate. Therefore, to investigate further the roles of phosphate and PTH in nephrocalcinosis in rats fed a basal diet containing alkali-treated soy protein, we studied the effects of PTX on the development of nephrocalcinosis.

Materials and Methods

Thirty-two female Sprague-Dawley rats weighing 125-165 g were used. Each animal was fasted for 12 hours and then anesthetized by an intraperitoneal injection of sodium pentobarbital (3.6 mg/100 g body weight). A midsagittal incision was made through the skin and investing fascia on the ventral surface of the neck. We retracted the infrahyoid muscles and incised the pretracheal fascia to expose the thyroid glands. In 16 of the animals, the parathyroid glands were identified and carefully excised by thermocoagulation. In the remaining 16 animals (which

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underwent sham operations) small thermocaustery lesions were made in the lobes of the thyroid gland, at a distance from the parathyroid glands. The wounds were then closed by sutures. We assessed the success of PTX by measuring the concentration of serum calcium shortly after the rats had recovered from the anesthetic. The PTX rats had serum calcium levels of less than 7.0 mg/dl, while in the sham operation group serum calcium was between 9.8 and 10.4 mg/dl.

Groups of 4 PTX and 4 sham operation rats were placed on each of the following diets, the composition of which have been described previously: 1) an alpha protein (an alkali-treated soy protein) diet (Ca/Pi-1:0.7); 2) a phosphate-supplemented alpha protein diet (Ca/Pi-1:2); 3) a commercial laboratory diet (Ca/Pi-1:0.7); and 4) a phosphate-supplemented commercial laboratory diet (Ca/Pi-1:2). The ratios of calcium to phosphorus for each of the diets presented above are approximations. The animals were maintained on the diets for 4 weeks, during which time they were housed individually in stainless steel cages and were allowed food and water ad libitum. Once each week, the animals were weighed, and a 24-hour food consumption measurement was made.

On the last day of the study the animals were anesthesized with ether, and their kidneys were removed. The left kidneys were cut into slices, which were processed for light and transmission electron microscopy, as described previously. The right kidneys were processed for ashing in a muffle furnace. In order to determine the efficacy of PTX, we removed the tracheas with the attached thyroid glands from the PTX rats, fixed them in 10% neutral buffered formalin, and processed them for light microscopic study.

Determinations of the levels of calcium in the serum and kidney ash samples were made with the use of a Varian Atomic Absorption Spectrophotometer. The phosphorus contents of the kidney ash samples were determined with the use of the Phosphorus Auto/Stat Kit (Pierce Chemical Co., Rockford, Ill) spectrophotometric method.

The quantitative results of the groups fed the alpha protein diets and the groups fed the commercial laboratory diets were analyzed statistically with two-way analysis of variance, followed by least significant difference multiple comparison tests.

Results

In the 4-week experimental period there were no overall differences between all the groups with respect to food consumption and weight gain.

There were no histologic evidence of residual parathyroid tissue in or associated with the thyroid glands of the PTX rats.

Morphology

Commercial Laboratory Diet

The kidneys of the sham operation and PTX rats fed the commercial laboratory diet appeared normal.

Phosphate-Supplemented Commercial Laboratory Diet

In the sham operation group fed the phosphate-supplemented commercial laboratory diet nephrocalcinosis was quite severe. Many intraluminal deposits were found at the junction of the outer and inner stripes of the outer medulla, in the inner stripe of the outer medulla, and in the inner medulla. In addition, evidence of severe tubular necrosis, interstitial fibrosis, inflammation, and foreign-body giant cell reaction were found, particularly in regions of heavy calcification. Ultrastructurally, intraluminal deposits were found primarily in pars recta segments of proximal tubules at the junction of the outer and inner stripes of the outer medulla and in collecting ducts and descending thin limbs of Henle in the inner stripe of the outer medulla. Some were also found in the ascending thick limbs of Henle in the inner stripe. In the inner medulla deposits were observed in the thin limbs of Henle and collecting ducts. Many of the cells around the calciferous deposits contained apatitelike crystals and had electron-dense membranes. Moreover, laminated deposits were found in the region of the basal lamina of collecting ducts and some tubules that could not be identified due to severe necrosis.

No evidence of renal calcification was found in the PTX rats fed the phosphate-supplemented commercial laboratory diet. In fact, the kidneys appeared normal both histologically and ultrastructurally.

Alpha Protein Diet

Several calciferous deposits were found in the inner medulla of the kidneys of both sham operation and PTX rats fed the basal alpha protein diet. No other structural abnormalities were observed in the kidneys of these animals.

Phosphate-Supplemented Alpha Protein Diet

In the sham operation rats fed the phosphate-supplemented alpha protein diet, nephrocalcinosis was also quite severe (Figure 1), in fact somewhat greater than that found in the sham operation group fed the phosphate-supplemented commercial labora-
Parathyroidectomy and Nephrocalcinosis

Figure 1—Transverse section of a kidney from a sham operation rat fed a phosphate-supplemented alpha protein diet for 4 weeks. A prominent wide band of calcification (black) is present at the junction of the outer and inner stripes of the outer medulla (OS/IS) and cortex (C). A number of calciferous deposits are also present in the inner stripe of the outer medulla (IS) and in the inner medulla (IM). (Alizarin red S, x20)

Figure 2—Calciferous deposits (black) in the inner medulla (IM) of a kidney from a PTX rat fed a phosphate-supplemented alpha protein diet for 4 weeks. (Alizarin red S, x70)

tory diet. The general histologic and ultrastructural features of nephrocalcinosis, however, were similar in the two groups.

A mild form of nephrocalcinosis was detected in the PTX rats fed the phosphate-supplemented alpha protein diet (Figure 2). The severity of the calcification and the localization of the deposits were the same as that found in the sham operation and PTX rats fed the basal alpha protein diet. No other changes were detected in the kidneys of these animals.

Kidney Ash Results

Commercial Laboratory Diets

Measurements of the calcium and phosphorus contents of the kidney ash gave results that supported the histologic findings (Tables 1 and 2). The calcium and phosphorus contents of the kidneys of both sham operation and PTX rats fed the commercial laboratory diet were about the same. Phosphate supplementation of the commercial laboratory diet resulted in a remarkable increase in the calcium and phosphorus contents of the kidneys of the sham operation rats. Parathyroidectomy, however, prevented these increases from occurring.

Alpha Protein Diets

The calcium and phosphorus contents of the kidneys from the sham operation group fed the basal alpha protein diet were about twice as great as those of the kidneys from the sham operation group fed the commercial laboratory diet. Parathyroidectomy had no effect on renal calcium and phosphorus in rats fed this basal diet. Phosphate supplementation of the alpha protein diet, however, resulted in a very large increase in renal calcium and phosphorus in sham operation rats. PTX rats fed the phosphate-supplemented diet had levels of renal calcium and phosphorus similar to those found in the sham operation and PTX groups fed the basal diet.

Discussion

The morphologic and kidney ash results of the present study showed that PTX completely prevented
the occurrence of nephrocalcinosis in female rats fed a phosphate-supplemented commercial laboratory diet. These results are consistent with those of Lehr and Krukowski\textsuperscript{11} and Clark and Rivera-Cordero,\textsuperscript{12} who found that phosphate-induced nephrocalcinosis was prevented in adult male rats by performing PTX. It is therefore apparent that PTH does play an important role in nephrocalcinosis induced by the ingestion of excess phosphate. The exact action(s) of the hormone that results in renal damage, however, is not yet known. It is probable, though, that PTH in conjunction with elevated levels of phosphate in the renal tubular fluid and/or the peritubular blood cause changes in the biochemical and physiologic activities in renal epithelial cells, which eventually lead to the deposition of calcium salts in the renal parenchyma. There are some findings from \textit{in vitro} studies that support this notion. It has been found that PTH causes a reduction in the respiratory activity of, and causes an increased uptake of calcium and phosphate by, mitochondria of cultured epithelial cells.\textsuperscript{13-15} In addition, it has been shown that when excess phosphate is added to the culture medium, there is an increased uptake of calcium by renal epithelial cells and their mitochondria.\textsuperscript{14} It must be kept in mind, however, that cultured renal epithelial cells do not necessarily reflect the same activities of renal epithelial cells \textit{in vivo}.

Parathyroid hormone, in pharmacologic doses, has been found to induce renal lesions in rats\textsuperscript{16} and mice.\textsuperscript{17} The lesions were found mainly in the proximal tubules, which are also known to be sites where the actions of PTH are mediated by an adenylate cyclase-cyclic AMP system. In the present study, however, many of the tubular segments where lesions were found in the kidneys of rats fed phosphate-supplemented diets, such as the medullary segments of the ascending thick limbs of Henle, the descending thin limbs of Henle, and the collecting ducts, are not thought to be sites where a PTH-dependent adenylate cyclase-cyclic AMP system is present.\textsuperscript{18-21} Given that PTH does play a role in dietary phosphate-induced nephrocalcinosis, and that the known effects of PTH are mediated by an adenylate cyclase system found almost exclusively in cortical tubules, it is possible that PTH, under conditions created by the ingestion of excess phosphate, has some toxic effect on the medullary tubules that is not mediated by a PTH-dependent adenylate cyclase-cyclic AMP system. It is also possible that PTH acts only on cortical tubules, but that under the conditions created by dietary phosphate loading the hormone causes the cortical tubules to produce some factor(s) and/or environmental condition that express(es) an effect on the medullary tubules that results in the deposition of calcium salts in the renal parenchyma.

Although PTX greatly reduced the amount of calcification in the kidneys of rats fed the phosphate-supplemented alpha protein diet, it did not completely prevent nephrocalcinosis from occurring. A few deposits were still found in the inner medulla. A similar number of deposits were found in the inner medulla of the kidneys of PTX and sham operation rats fed the unsupplemented alpha protein diet. The

| Table 1 — Total Renal Calcium and Phosphorus (Pi) in Sham Operation and PTX Rats Fed a Phosphate-Supplemented and Unsupplemented Commercial Laboratory Diet for 4 Weeks |
|---------------------------------|-----------------|
|                                 | Renal calcium  | Renal Pi        |
|                                 | (mg/g,* mean ± SD) | (mg/g,* mean ± SD) |
| Sham operation, commercial laboratory diet | 0.5 ± 0.1† | 5.9 ± 1.0† |
| PTX, commercial laboratory diet | 0.4 ± 0.1† | 5.5 ± 0.3† |
| Sham operation, commercial laboratory diet + phosphate | 12.7 ± 1.1 | 30.6 ± 12.3 |
| PTX, commercial laboratory diet + phosphate | 0.5 ± 0.1 | 5.9 ± 0.1 |

* Based on fat-free dry kidney weight.
† Statistically different (P < 0.05) from the mean of the sham operation group fed the phosphate-supplemented commercial laboratory diet.

| Table 2 — Total Renal Calcium and Phosphorus (Pi) in Sham Operation and PTX Rats Fed a Phosphate-Supplemented and Unsupplemented Alpha Protein Diet for 4 Weeks |
|---------------------------------|-----------------|
|                                 | Renal calcium  | Renal Pi        |
|                                 | (mg/g,* mean ± SD) | (mg/g,* mean ± SD) |
| Sham operation, alpha protein diet | 1.1 ± 0.3† | 14.3 ± 0.3† |
| PTX, alpha protein diet | 0.9 ± 0.1† | 14.3 ± 1.2† |
| Sham operation, alpha protein + phosphate diet | 14.0 ± 3.4 | 32.8 ± 6.4 |
| PTX, alpha protein + phosphate diet | 0.9 ± 0.1† | 13.8 ± 0.7† |

* Based on fat-free dry kidney weight.
† Statistically different (P < 0.05) from the mean of the sham operation group fed the phosphate-supplemented alpha protein diet.
total calcium and phosphorus contents of the kidneys from the animals in these three groups were also similar and were about twice as great as those of the kidneys from the rats of the corresponding groups fed the commercial laboratory diets, thus supporting the morphologic findings. It should also be mentioned that the characteristics of nephrocalcinosis, including severity and localization of deposits, in the sham operation group fed the basal alpha protein diet for 4 weeks were very similar to those in rats fed the same diet for 3 weeks in our previous study. Therefore, it appears that the nephrocalcinosis in the PTX rats fed the basal alpha protein or phosphate-supplemented alpha protein diet was not induced by PTH or by excess phosphate, but was induced by some other factor(s) associated with the basal alpha protein diet, which remain(s) unknown at the present time.

The results of the present study suggest that nephrocalcinosis induced in rats by excess dietary phosphate is actually mediated by PTH. In addition, the results suggest that the nephrocalcinosis in rats fed a basal diet containing alpha protein is induced neither by PTH nor by excess dietary phosphate.

References


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