INTESTINAL CALCIUM AND PHOSPHATE TRANSPORT IN GENETIC HYPOPHOSPHATEMIC MICE[+]

by

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Serum phosphate, serum calcium, intestinal phosphate and intestinal calcium transport were measured in normal (C57BL/6J +/Y) and genetic (X-linked) hypophosphatemic mice (C57BL/6J Hyp/Y). The hypophosphatemic mice had low serum phosphorus levels and dramatically decreased intestinal phosphate transport compared with normal controls. On the other hand, normal and hypophosphatemic mice had equivalent levels of intestinal calcium transport. The hypophosphatemic mice did illustrate a slightly decreased serum calcium concentration, however. Administration of 1,25-dihydroxy-vitamin D_3, the principal active metabolite of vitamin D, stimulated intestinal calcium transport but not intestinal phosphate transport in the genetic hypophosphatemic mice. The results obtained are consistent with the hypothesis that the primary metabolic disturbance in familial hypophosphatemia involves a defect in phosphate transport mechanisms.

A metabolic bone disorder known as familial hypophosphatemic vitamin D resistant rickets is well known to clinicians (1). This genetic disease has an X-linked dominant inheritance and is characterized by low serum phosphorus, normal or slightly low serum calcium, and high urinary phosphorus with a reduced renal reabsorption of phosphorus (1). Serum immunoreactive parathyroid hormone levels may be low (2), normal (3) or elevated (4, 5) and intestinal calcium absorption is below normal (1). The metabolic basis for the disease is unknown, and theories include a defect in vitamin D metabolism (1), an increased sensitivity to parathyroid hormone (6) and a defect in tubular reabsorption of phosphate (7). That the disease is a generalized

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defect in phosphate transport reactions has also been put forth based on the observation of reduced phosphate uptake by intestinal biopsy samples taken from hypophosphatemic patients (8). However, a detailed investigation of this disease has been severely hampered by the lack of a suitable animal model.

Recently an X-linked dominant mutation was discovered in the laboratory mouse that causes hypophosphatemia and rickets (9) similar to the human disease. We report here that hypophosphatemic mice (Hyp/Y) show a markedly reduced transport of phosphate in intestine, while exhibiting normal intestinal calcium transport. Thus, in mouse X-linked hypophosphatemia, a generalized defect in phosphate transport reactions may be the primary disturbance giving rise to the phenotypic manifestations of the disease.

MATERIALS AND METHODS

C57BL/6J +/Y males (normal) and C57BL/6J Hyp/Y males were bred at the Bar Harbor Laboratories. The animals were maintained in Madison, Wisconsin, in overhanging wire cages with food and water supplied ad libitum. They were fed a laboratory mouse diet (1.2% calcium, 0.99% phosphorus) (Wayne Lab Blox) for four weeks. The animals were fourteen weeks old when used for experimentation. The intestinal calcium transport measurement was carried out with duodena prepared as described by Martin and DeLuca (10). Intestinal phosphate transport was measured in jejuna by the procedure of Chen et al. (11). Blood, obtained by decapitation, was centrifuged to yield serum that was used for the determination of calcium in 0.1% $LaCl_3$ with an atomic absorption spectrometer and inorganic phosphorus by the method of Chen et al. (12). 1,25-Dihydroxyvitamin D_3 ($1,25\text{-}(OH)_2D_3$)[1] was chemically synthesized as previously described (13).

RESULTS AND DISCUSSION

Table 1 shows that the serum calcium concentration of the genetic hypo-

[1]Abbreviations: $1,25\text{-}(OH)_2D_3$, 1,25-dihydroxyvitamin D_3.

Table 1. Serum Calcium and Phosphorus Concentration in Normal and Hypophosphatemic Mice

Strain	Genotype	Serum Calcium (mg/100 ml)	Serum Phosphorus (mg/100 ml)
C57BL/6J	+/Y	10.2 ± 0.5	7.6 ± 0.5
C57BL/6J	Hyp/Y	8.7 ± 0.4	2.3 ± 0.3

The data are reported as the mean ± SEM. There were 5 mice in each group.

phosphatemic mice is slightly lower than normal while the serum phosphorus level is extremely low. In this respect the mutant mice are similar to hypophosphatemic man having the X-linked dominantly inherited disease. In addition, the hypophosphatemic mice show boney lesions of rickets (9).

Intestinal calcium and phosphate transport in the normal and hypophosphatemic mice are shown in Table 2. The hypophosphatemic mice exhibit markedly suppressed intestinal phosphate transport, while calcium transport is normal. In addition, the administration of $1,25\text{-}(OH)_2D_3$ did not alter the low intestinal phosphate transport in the hypophosphatemic mice while it did stimulate calcium transport. In contrast to this, both intestinal transport systems were stimulated in the normal mice.

In the genetic hypophosphatemic mouse, therefore, it appears that a primary disorder is defective intestinal phosphate transport, which may reflect a generalized phosphate transport disorder. Furthermore, the fact that the active form of vitamin D will not correct the phosphate transport defect supports the idea that this disease is not the result of defective vitamin D metabolism. These results also support the previous conclusion that the human disease is associated with defective intestinal phosphate transport (8). Moreover, these results suggest that the hypophosphatemic mice may represent

Table 2. Intestinal Calcium and Phosphate Transport in Normal and Hypophosphatemic Mice

Strain	Genotype	Ng of $1,25\text{-}(OH)_2D_3$ Given	Intestinal Transport: Calcium — Serosal ^{45}Ca/ Mucosal ^{45}Ca	Intestinal Transport: Phosphate — Serosal ^{32}P/ Mucosal ^{32}P
C57BL/6J	+/Y	0	5.2 ± 0.6	6.8 ± 0.8
		25	6.7 ± 0.8	7.7 ± 0.9
		50	7.9 ± 0.6	8.9 ± 1.6
		75	8.4 ± 0.8	12.6 ± 2.9
C57BL/6J	Hyp/Y	0	4.8 ± 0.5	2.4 ± 0.4
		25	6.2 ± 0.6	2.5 ± 0.5
		50	7.7 ± 0.8	2.5 ± 0.6
		75	8.2 ± 0.7	2.6 ± 0.6

The data represent the average determination from 5 animals ± SEM. Intestinal calcium transport was measured in proximal duodenum (10) and phosphate in the jejunum (11). Where appropriate, mice were given $1,25\text{-}(OH)_2D_3$ intraperitoneally dissolved in 0.05 ml of ethanol. Controls received the vehicle alone.

a valid model of the human disease, giving investigators an important probe in the pursuit of the metabolic basis of the disease.

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