Vol. 100, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1981 Pages 537-543

INTESTINAL TRANSPORT OF PHOSPHATE ANION IS NOT IMPAIRED IN THE *Hyp* (Hypophosphatemic) MOUSE[+]

by

Harriet S. Tenenhouse, Diane K. Fast, Charles R. Scriver and Magdolna Koltay

De Belle Laboratory for Biochemical Genetics and Medical Research Council Genetics Group, McGill University-Montreal Children's Hospital Research Institute, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3

Received February 23,1981

SUMMARY: Intestinal transport of ^{32}P-labelled inorganic phosphate was measured in everted gut sacs, and scraped enterocytes prepared from segments along the length of small intestine, from duodenum to the terminal ileum of hypophosphatemic male (*Hyp*/Y) mice and normal (+/Y) littermates. Uptake of phosphate by purified brush-border membrane vesicles prepared from the small intestine of both genotypes was also measured. We found no deficiency of phosphate transport in the *Hyp* small intestine. In vitro transport of phosphate by sacs and enterocytes is greatest in the distal intestinal segments of mouse.

The *Hyp* mouse is a homologue of XLH in man (1). The X-linked dominant murine phenotype is characterized by chronic hypophosphatemia, rickets, and a defect in renal tubular reabsorption of phosphate (1). Tenenhouse et al (2,3) demonstrated a selective loss of Pi transport in renal brush-border membrane vesicles prepared from *Hyp* kidney. O'Doherty et al (4,5) reported that intestinal transport of Pi, in the everted gut sac preparation, was also impaired in the *Hyp* phenotype and was hyporesponsive to 1,25-dihydroxy-vitamin D_3.

Because intestinal and renal epithelia share a common embryological origin, and also have many topological and functional aspects in common, we presumed the transport defect in the *Hyp* phenotype would be apparent in the

[+] This work was supported by the Medical Research Council of Canada

Abbreviations used: Pi, phosphate anion; *Hyp*, X-linked hypophosphatemia (murine); XLH, X-linked hypophosphatemia (human)

0006-291X/81/100537-07$01.00/0

intestinal brush-border membrane, as it is in the renal brush-border membrane. We report here our inability to show a deficiency of Pi uptake in Hyp intestinal brush-border membrane vesicles and enterocytes prepared from sites along the full length of small intestine. On re-examination of transmural transport by everted gut sacs, from duodenum to terminal ileum, we were unable to confirm the finding, reported by O'Doherty and colleagues of deficient Pi transport in the Hyp mouse intestine.

MATERIALS AND METHODS

Normal (+/Y) and hemizygous (Hyp/Y) male littermates were raised in our laboratory. The initial breeding pairs, obtained from the Jackson Laboratory, were taken from the original Hyp stock used by O'Doherty et al (4,5). The animals were fed lab Chow (Ralston Purina of Canada, Ltd) and were at least 5 months old at the time of sacrifice. Intestinal brush-border membrane vesicles were prepared from jejunum and upper ileum by the method of Schmitz et al (6). Membrane purity was monitored by enzyme assays, and uptake of Pi and D-glucose was measured in brush-border membrane vesicles by the Millipore filtration technique, as described previously (3). Everted gut sacs were prepared by a modification of the method of Chen et al (7). We divided the total small intestine, from pylorus to caecum, into 3 equal segments; after eversion, 3 sacs of 3-4 cm each were prepared from each segment. Sacs were numbered, one to nine, from duodenum to terminal ileum, respectively. All manipulations were carried out rapidly at 4°C. The gut sacs were filled with pH 7.4 buffer containing 100 mM NaCl, 18 mM KCl, 1 mM K_2HPO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 2.5 mM glutamine, 0.5 mM β-hydroxybutyrate and 20 mM Tris-Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid). Each sac was then incubated in gassed buffer (10 ml) for 60 min at 37°C under 100% O_2 in 25 ml Erhlenmeyer flasks. Labelled substrate (^{32}P, 0.4 μC/ml) was added to the flask at zero time. After the incubation, the sacs were rinsed in ice-cold saline and blotted. Aliquots of the serosal contents of each sac and the incubation medium were counted and the serosal-to-mucosal (S/M) distribution ratios calculated. [^3H]-polyethylene glycol was also placed in the serosal cavity or in the medium to monitor sac integrity.

Intestinal rings (2 cm width) were prepared in chilled buffer and incubated with ^{32}Pi or [^3H]-polyethylene glycol (8) for 60 min at 37°C. The incubation buffer was that used for gut sacs. At the end of incubation, rings were removed, rinsed, blotted, and scraped to obtain enterocytes (8). The scrapings were dried at 55°C for 100 min, weighed, digested in Protosol, and counted. We calculated and subtracted the contribution from the extra-cellular space and expressed uptake of ^{32}Pi by enterocytes as nmoles Pi/mg dry wt.

Carrier-free ^{32}P (Cat. no. NEX-054), polyethyleneglycol (Cat. no. NET-405), D-[^{14}C]-glucose (Cat. no. NET-042X), and Protosol were purchased from New England Nuclear, Boston, Mass. Hepes, glutamine and β-hydroxybutyrate were obtained from Sigma Chemical Co., St. Louis, MO and Millipore filters, type HA, 0.45 μm, from Millipore Corporation, Bedford, MA. Scintiverse, a scintillation fluid, and all common reagents were bought from Fisher Scientific, Montreal Canada.

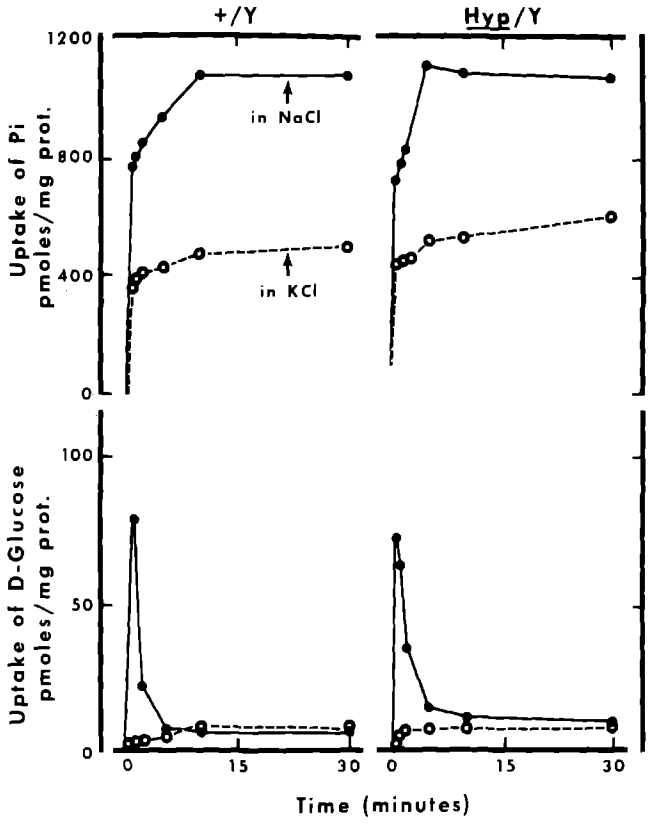

Figure 1: Uptake of ^{32}Pi and ^{14}C-D-glucose by brush-border membrane vesicles (BBMV) prepared from upper small intestine of +/Y and Hyp/Y mice. There are no significant differences in uptakes of labelled Pi and glucose by intestinal BBMV prepared from +/Y and Hyp/Y mice.

RESULTS AND DISCUSSION

We achieved 10-fold enrichment of brush-border membrane sucrase activity in our intestinal vesicle preparation from +/Y and Hyp/Y mice. When we increased the osmolarity of the medium with cellobiose, an impermeable sugar, we determined that Pi uptake represented transport into an osmotically sensitive intravesicular volume (data not shown). Time courses for uptake of phosphate and glucose by intestinal brush-border membrane vesicles, prepared from normal and Hyp mice, were carried out in the presence of KCl and NaCl gradients (Figure 1). Diffusional uptakes (in KCl) and Na^+

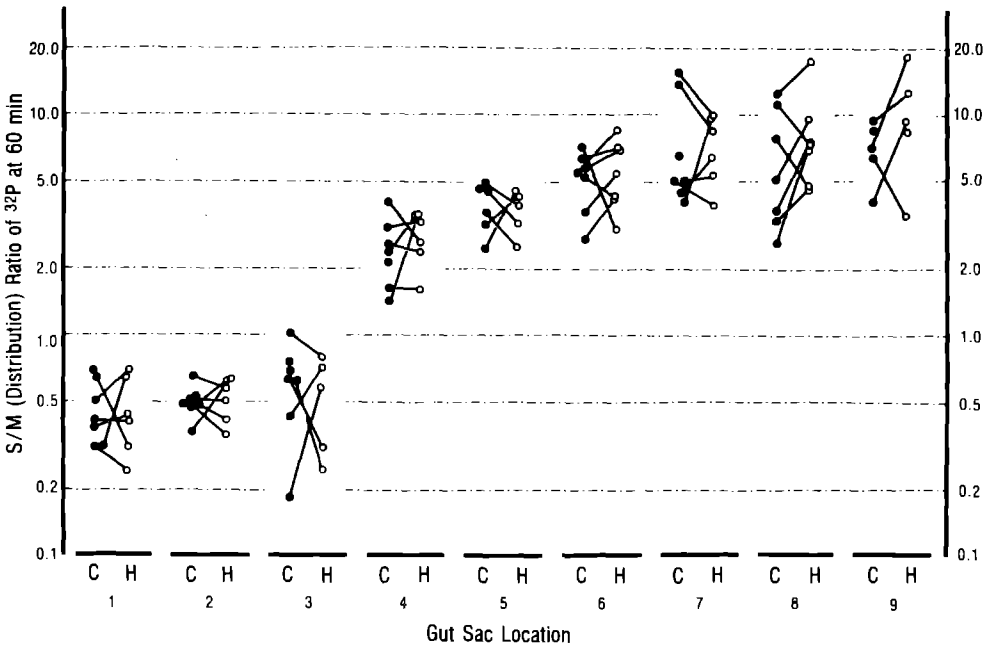

Figure 2: Serosal-to-mucosal (S/M) distribution ratios in everted gut sacs from control (●) and Hyp (○) mice. Segments 1-9 represent sequential regions from duodenum to terminal ileum, respectively. Paired data of +/Y and Hyp/Y sacs incubated on the same day are shown by joining lines in graph. Data were analyzed by two-way Anova; there are no significant differences between Hyp/Y and +/Y uptakes of ^{32}Pi.

gradient-dependent uptakes of Pi and glucose were both similar, for normal and Hyp mice in intestinal brush-border membrane vesicles. This finding has been confirmed in another laboratory with +/Y and Hyp/Y mice from our laboratory (B. Sacktor, personal communication, 1980). Accordingly, the defect in Pi transport, observed in the renal brush-border membrane of Hyp mice (2,3), is not found in the corresponding brush-border membrane of small intestine.

The S/M distribution ratio for ^{32}Pi in the everted gut sac preparation is shown for +/Y and Hyp/Y genotypes in Figure 2. Transport of ^{32}Pi was comparable in all segments along the small intestine of Hyp and normal mice. S/M distribution ratios for sacs incubated in Krebs-Ringer buffer (the buffer used by O'Doherty et al (4,5)) were much lower, but

TABLE 1. UPTAKE OF ^{32}Pi BY ENTEROCYTES OF SMALL INTESTINE FROM NORMAL (+/Y) AND HYPOPHOSPHATEMIC (Hyp/Y) MICE.

^{32}Pi Uptake, nmole/mg dry wt · 60 min (mean ± SEM, n = 5)		
Region	+/Y	Hyp/Y
1	4.9 ± 1.7	4.8 ± 0.9
2	8.5 ± 4.3	5.9 ± 1.1
3	10.9 ± 4.6	7.8 ± 1.6
4	24.7 ±10.7	16.5 ± 2.4
5	20.6 ± 7.0	26.5 ± 6.2
6	20.2 ± 6.1	26.9 ± 5.1
7	36.8 ± 7.5	44.5 ± 8.9
8	25.8 ± 7.7	33.7 ± 4.0
9	24.3 ± 2.6	29.3 ± 3.2

Intestinal rings were prepared from segments along the entire length of small intestine (1, proximal duodenum; 9 terminal ileum) as described for Figure 2. After incubation enterocytes were scraped and analyzed as described. Two-way Anova for 9 regions in five experiments: +/Y vs Hyp/Y, NS.

were not different in Hyp and normal mice (data not shown). Accumulation of ^{32}Pi by intestinal enterocytes was also similar in Hyp/Y and +/Y mice (Table 1). Finally, we found no difference in the *in situ* tissue Pi concentration in the two genotypes (data not shown).

Intestinal uptake of ^{32}Pi *in vitro* in mouse intestine was higher in distal segments when compared with proximal segments (Figure 2 and Table 1). The pattern for Pi transport is similar to that reported for sulfate in mouse small intestine (9). The pattern differs from that described for Pi transport in rat small intestine; we have confirmed the earlier report (10) that maximal uptake of phosphate occurs in segments near the midpoint of rat small intestine.

Our inability to demonstrate a deficiency of intestinal phosphate transport in the Hyp mouse, as originally reported (4,5), concerns us. We used three different preparations of intestinal tissue to confirm our findings. We also incubated the everted sacs under two different conditions: those used previously (4,5) and in a medium with improved buffering capacity for Pi transport that contained nutrients for intestinal tissues (11). Although our incubations were shortened to 60 min to minimize tissue breakdown during these experiments, we could still find no abnormality with the 90-min incubation period reported by O'Doherty et al (4,5) (data not shown). We also examined Pi transport in sacs and enterocytes along the whole length of the small intestine to avoid any ambiguity about the segment of gut studied.

Short et al (12) found deficient Pi uptake in jejunal biopsies from XLH patients; on the other hand, Glorieux et al (10) found no abnormality. The reason for this discrepancy is not understood but may be due to genetic heterogeneity of the patients studied in the two groups. The finding reported by Short et al (12) might reflect a slightly attenuated enterocyte uptake of phosphate in the proximal small intestine of XLH patients that is analogous to the finding in the Hyp intestine (Table 1). Whatever the explanation for this finding, it is not the result of deficient phosphate transport at the brush-border membrane in this portion of small intestine.

We have proposed that the primary phenotype in the Hyp mouse is a specific defect in Pi transport confined to the renal brush-border membrane (2,3). Since human XLH involves the loss of a renal Pi transport system sensitive to parathyroid hormone (14), one would expect the gut, which lacks such a system, not to be affected in the human phenotype. Our findings in the Hyp mouse, a homologue of human XLH (1), offer a clarification of the human phenotype and are relevant to the interpretation of treatment procedures that attempt to restore Pi homeostasis in the mutant phenotype.

REFERENCES

1. Eicher, E.M., Southard, J.L., Scriver, C.R. and Glorieux, F.H. (1976) Proc. Natl. Acad. Sci. USA 73, 4467-4671.

2. Tenenhouse, H.S., Scriver, C.R., McInnes, R.R. and Glorieux, F.H. (1978) Kidney Int. 14, 236-244.

3. Tenenhouse, H.S. and Scriver, C.R. (1978) Can. J. Biochem. 56, 640-646.

4. O'Doherty, P.J.A., DeLuca, H.F. and Eicher, E.M. (1976) Biochem. Biophys. Res. Commun. 71, 617-621.

5. O'Doherty, P.J.A., DeLuca, H.F. and Eicher, E.M. (1977) Endocrinology 101, 1325-1330.

6. Schmitz, J., Preisner, H., Maestracci, D., Ghosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98-112.

7. Chen, J.C., Castillo, L., Korycka-Dahl, M. and DeLuca, H.F. (1974) J. Nutr. 104, 1056-1060.

8. Nutzenadel, W. and Scriver, C.R. (1976) Amer. J. Physiol. 230, 643-651.

9. Batt, E.R. (1969) Am. J. Physiol. 217, 1101-1104.

10. Harrison, H.E. and Harrison, H.C. (1961) Am. J. Physiol. 201, 1007-1012.

11. Kimmich, G.A. and Randles, J. (1979) Am. J. Physiol. 237, C56-C63.

12. Short, E.M., Binder, H.J. and Rosenberg, L.E. (1973) Science 179, 700-702.

13. Glorieux, F.H., Morin, C.L., Travers, R., Delvin, E.E. and Poirier, R. (1976) Pediat. Res. 10, 691-696.

14. Glorieux, F. and Scriver, C.R. (1972) Science 175, 997-1000.