Effect of the X-linked Hyp Mutation on N-Ethylmaleimide Labelling of Proteins in Renal Brush Border Membrane

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Summary: The X-linked dominant mutation, hypophosphataemia (gene symbol, Hyp) is expressed in the laboratory mouse as deficient phosphate transport at the renal brush border membrane (BBM) of proximal nephron. In an attempt to identify proteins which mediate phosphate transport, we treated renal BBM vesicles prepared from mutant male (Hyp/Y) and normal male (+/Y) littermates, with radiolabelled N-ethylmaleimide (NEM), in the presence or absence of arsenate which is a competitive inhibitor of phosphate transport. Polyacrylamide gel electrophoresis revealed labelling of membrane proteins in the 40-45 kDa range; addition of arsenate during NEM treatment inhibited labelling. These findings indicate a 40–45 kDa protein as a component of the renal BBM phosphate transport system(s). We found no difference between protein labelling of the renal BBM from Hyp/Y and +/Y mice.

Renal reabsorption of filtered phosphate is an important component in the regulation of phosphate homeostasis in the mammal (Scriver and Tenenhouse, 1981) and impairment of the renal transport process perturbs phosphate homeostasis. Mutations which cause specific deficiencies of renal tubular reabsorption of phosphate anion identify genes that designate the process and by inference the polypeptide translation products essential to it. Such genes are present on both the X-chromosome and an unspecified autosome in man and were identified through the study of Mendelian phenotypes (Scriver and Tenenhouse, 1981). Analogous studies in the mouse reveal two loci, Hyp and Gy, on the X-chromosome (Eicher et al., 1976; Lyon et al., 1986) which determine phosphate transport at the renal brush border membrane (Tenenhouse et al., 1978; Tenenhouse and Scriver, 1978; Lyon et al., 1986). In the present study we describe an initial attempt to identify a protein or proteins which mediate(s) phosphate transport in mouse renal brush border membranes. We treated membrane vesicles with radiolabelled N-ethylmaleimide

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(NEM) in the presence or absence of arsenate, the latter being a competitive inhibitor of phosphate transport in renal brush border membrane vesicles (Hoffman et al., 1976). We used polyacrylamide gel electrophoresis to examine the pattern of protein labelling. We also performed studies to examine the effect of the Hyp mutation (Eicher et al., 1976) on NEM labelling of mouse renal brush border membrane proteins. We found a 40–45 kDa membrane protein whose labelling was inhibited by arsenate. We found no differences between the labelling of Hyp and control membranes.

MATERIALS AND METHODS

Materials

Mice: We used C57B1/6J normal males (genotype +/Y) and hemizygous mutant (Hyp/Y) littermates in these experiments. The initial breeding pairs (+/Y males and Hyp/+ females) were obtained from the Jackson Laboratory, Bar Harbour, ME, USA and from R. A. Meyer, Marquette University, Milwaukee, WI, USA. Breeders were maintained on Wayne Breeder-Blox diet (Allied Mills Inc., Chicago, IL, USA) which contains 1.2% (w/w) calcium, 0.99% phosphorus, and 4.41 IU vitamin D g⁻¹. Mice were under one year of age at sacrifice.

Chemicals: [³H]-N-ethylmaleimide, [¹⁴C]-N-ethylmaleimide and Formula 963 scintillation fluid were purchased from New England Nuclear (Lachine, Quebec, Canada). Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were obtained from Bio-Rad Laboratories (Richmond, CA, USA). Filters (0.45 μm, type HA) were obtained from Millipore Corporation (Bedford, MA, USA); N-ethylmaleimide from Eastman Kodak (Rochester, NY, USA); and Cronex-4 film for autoradiography from BDH chemicals (Toronto, Canada). All other chemicals were obtained from Sigma Chemicals Co. (St Louis, MO, USA) and Fisher Scientific Ltd. (Montreal, Quebec, Canada).

Methods

Renal brush border membrane vesicles: brush border membrane vesicles were prepared from kidney cortex by the divalent cation precipitation method (Booth and Kenny, 1974). Enrichment of brush border membranes was tenfold as assessed by alkaline phosphatase activity. Protein concentration was determined by the Folin phenol method (Lowry et al., 1951).

Differential labelling of membranes: Our approach was based on the methods of Fox and Kennedy (1965) as modified by Lemaire and Maestracci (1978). Two cycles of NEM treatment were performed. In the first cycle, membranes were treated with non-radioactive NEM in the presence of arsenate, a competitive inhibitor of phosphate transport (Hoffmann et al., 1976), so that all sites reactive with NEM and not protected by arsenate reacted with non-radioactive NEM. In the second cycle, reaction of membranes with either [³H]-NEM or [¹⁴C]-NEM was carried out in the presence or absence of arsenate.