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DEVELOPMENTAL BIOLOGY REVIEW

Adrian Spitzer · Mario Barac-Nieto

Ontogeny of renal phosphate transport and the process of growth

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Abstract The kidneys of infants and children reabsorb a high fraction of the filtered phosphate (Pi), as appropriate to the needs of a growing organism. This high Pi reabsorptive rate is associated with a high capacity (V_{max}) of the Na^+-Pi symport system. At the molecular level this high reabsorptive capacity appears to be due to the presence of a growth-specific Na-Pi cotransporter. Several experimental findings support this assumption. Firstly, the expression of NaPi-2 mRNA is, if anything, lower in the renal cortex of young animals than of adult animals. Secondly, polyA RNA obtained from growing animals depleted of NaPi-2 by specific hybridization with an antisense 16-mer induces Na^+-Pi transport in oocytes. No induction of Na^+-Pi transport was observed in oocytes injected with hybridized polyA RNA obtained from adult animals. Thirdly, polyA RNA derived from young rats, depleted of NaPi-2 by subtractive hybridization with adult animal renal cortical cDNA, retains its ability to encode for Na^+-Pi cotransport in oocytes. Adult animal renal cortical polyA RNA, depleted of NaPi-2 by subtractive hybridization, failed to induce Na^+-Pi uptake into oocytes. Fourthly, renal cortical polyA RNA from young animals, depleted of NaPi-2, contains a region that is highly homologous (80%–92%) with the corresponding region of other modulated NaPi (type II) transporters. Fifthly, this region is also present in the polyA RNA obtained from the renal cortex of newborn rats (1st week of life), despite the fact that NaPi-2 is absent at this early age. Lastly, Npt2 (–/–) knockout mice, although hypophosphatemic and phosphaturic, filter and reabsorb Pi at rates exceeding those that can be accounted for by the expression of type I and III transporters. Based on these observations it is reasonable to surmise that the high V_{max} of the Na^+-Pi cotransport system observed in the young is due to a large extent to the presence of a growth-specific NaPi transporter, homologous but not identical to already cloned type II NaPi transporters.

Keywords Phosphate · Ontogeny · Renal transport · Mineral metabolism

A. Spitzer (✉)
Albert Einstein College of Medicine, Montefiore Medical Center, New York, NY 10467, USA
e-mail: spitzer@aecom.yu.edu

M. Barac-Nieto
Faculty of Medicine, Kuwait University, Safat 13110, Kuwait

Phosphate and the process of growth

Phosphate (Pi)is of critical importance to body functions, particularly during periods of growth. The phosphorylation-dephosphorylation cycle of ATP to ADP is the major energy currency of the cells. Pi forms the backbone of the desoxi- and ribonucleotides that constitute the genes and ribosomes. Protein phosphorylation-dephosphorylation cycles mediated by kinases are paramount to intracellular signaling. Pi is a major constituent of the phospholipids that form plasma and intracellular membranes, and thus determine cellular compartmentation and transport.

The body content of phosphorus increases from 4–5 g (approximately 0.16 mol)/kg in the newborn to 10–12 g (approximately 0.36 mol)/kg in the adult, reflecting the increasing proportions of mineralized bone and cellular tissues per unit of body mass [1]. Balance studies indicate that a 1- to 3-month-old infant fed a standard formula retains 32±25 (SD) mg/kg body weight of phosphate [2, 3], whereas the adult, by definition, retains none. The achievement of a positive external balance is dependent on intake, intestinal absorption, and the ability of the kidneys to reclaim filtered Pi. Dietary intake of Pi per unit body weight is more than two times higher in the newborn than in the adult. Little Pi is lost in the feces, reflecting its extensive absorption [4], a process stimulated by 1,25-dihydroxyvitamin D_3.

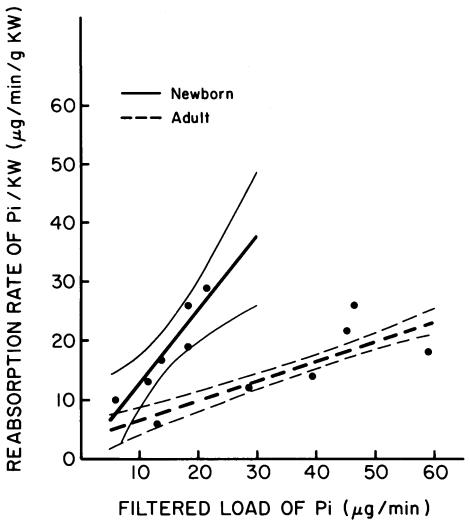

Fig. 1 Regression lines and 95% confidence limits of the relationship between the reabsorption of phosphate (*Pi*) and the filtered load of Pi by isolated perfused kidneys of newborn (y=1.25x+0.09) and mature (y=0.34x+3.1) guinea pigs (Johnson and Spitzer [10])

The contribution of the kidney to the positive Pi balance required for growth

The kidneys contribute to the maintenance of the positive Pi balance required for growth by reabsorbing a high fraction of the filtered Pi (99% in newborns, 95% in infants fed human milk, and 80% in adults) [5, 6, 7]. Experiments performed on dogs [8] and rats [9] provided suggestive evidence that this high reabsorptive capacity is intrinsic to the kidneys. That this is indeed the case was demonstrated by studies performed by Johnson and Spitzer [10] on isolated kidneys of guinea pigs perfused in vitro with solutions containing Pi in concentrations varying between 3 and 15 mg/dl. The slopes of the regression lines describing the relationship between the filtered load of Pi and the amount reabsorbed per unit of kidney weight (Fig. 1) illustrate that at any given filtered load of Pi the kidney of the newborn reabsorbed almost four times as much Pi than that of the adult.

The fact that the fractional reabsorption of Pi is higher in growing than in fully grown animals and humans was surprising when one considered age-related differences in the variables known at the time to govern tubular Pi reabsorption [11]. The surface area of the proximal tubule brush order is at least 100-fold smaller at birth than in adulthood [12, 13]. The change results from a 2.5-fold increase in tubular diameter (which causes a 6.5-fold increase in surface area), a 10-fold increase in tubular length (which accounts for a 10-fold increment), and an increase in the height of brush border villi of about 30% (which makes up the remaining 1.5-fold). The density of the microvilli also increases, but the contribution of this factor to the change in transporting surface area cannot be accurately assessed. In addition, the S1 segment of the proximal tubule, in which most of the

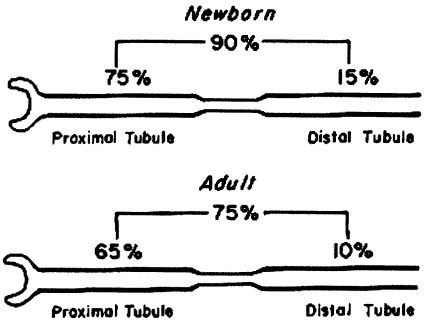

Fig. 2 Schematic representation of the changes in fractional reabsorption of Pi along the renal tubule during maturation. (Based on data from Kaskel et al. [23])

Pi is reabsorbed in the adult [14, 15], is poorly represented in the newborn [16]. Functional variables affecting Pi transport could not be invoked to compensate for the morphological immaturity. The fractional reabsorption of sodium and fluid along the proximal tubule does not change appreciably with age [17, 18], and thus Pi reabsorption due to solvent drag should also remain relatively constant. The flow rate through the proximal tubule of superficial nephrons increases in direct relation to single nephron glomerular filtration rate (GFR), but due to the larger increase in luminal surface area, the contact time per unit of membrane surface is about fourfold lower at birth than at maturity. The permeability of the proximal tubule to macromolecules is greater in the newborn than later in life [19] due to differences in tight junctions [20], rather than cellular membrane permeability characteristics [21, 22]. Thus, these variables, taken singly or in combination, could not account for the avid reabsorption of Pi observed in the newborn. However, micropuncture experiments performed by Kaskel et al. [23] revealed that at comparable locations along the proximal tubule the fraction of the filtered Pi reabsorbed was significantly higher in immature than in mature guinea pigs (Fig. 2). Eighty-five percent of the age-related difference in the renal reabsorption of Pi could be explained by higher rates of Pi reabsorption in the proximal segments of the nephrons, and the remaining 15% by differences in reabsorption at more distal nephron sites. It should be noted that this enhanced tubular reabsorption of Pi occurs despite the fact that the concentration of Pi in the extracellular fluid is very high at birth (5.8–9.3 mg/dl), and decreases slowly thereafter, to reach adult values (3.0–4.5 mg/dl) as late as the 2nd decade of life [3, 6].

Kinetics of Na^+-Pi cotransport during development

One possible mechanism underlying the high renal reabsorption of Pi observed during growth could reside in the Na^+-Pi cotransport system. Three factors need to be considered: (1) the turnover rate of the Pi carrier (V_{max}); (2) the affinity of the membrane carrier for Pi (K_m); and (3) the extra- to intracellular Pi concentration gradient. In

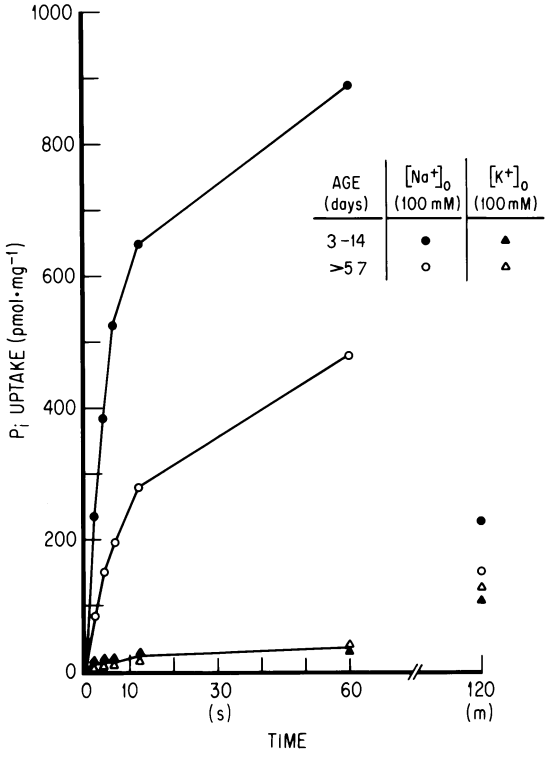

Fig. 3 Time course of Pi (0.1 mM) uptake in brush border membrane vesicles (*BBMV*) of 3- to 14-day-old and >57-day-old guinea pigs. *Circles*, uptake in the presence of 100 mM Na^+ gradient. *Triangles*, uptake in the presence of 100 mM inwardly directed KCl gradient. (From Neiberger et al. [24])

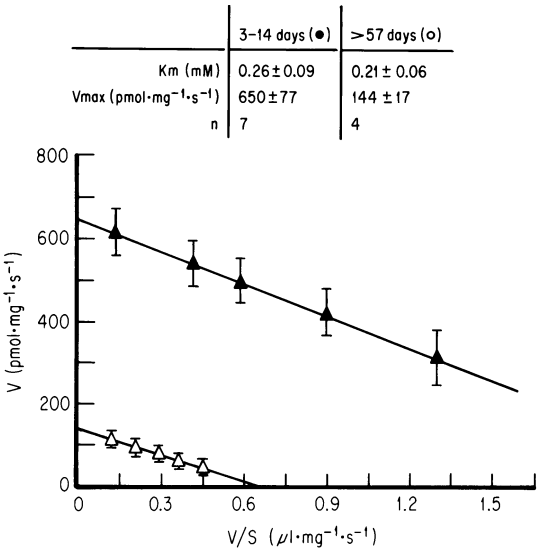

Fig. 4 Eddie-Hofstee plot of Na^+-dependent Pi influx into BBMV obtained from 3- to 4-day-old and >57-day-old guinea pigs on standard diet. Measurements were made at 2–6 s with an inwardly directed Pi gradient ranging from 0.1 to 4.0 mM. (From Neiberger et al. [24])

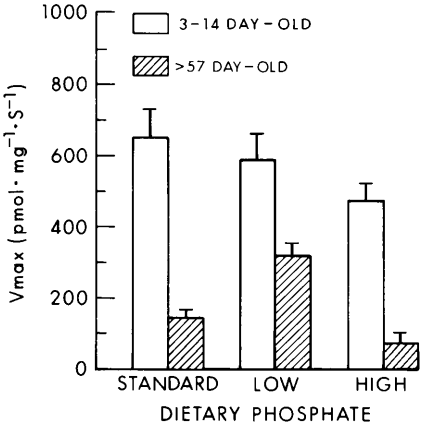

Fig. 5 The maximal velocity (V_{max}) of Na^+-Pi cotransport in brush border membranes of newborn (3- to 14-day-old) and adult (>57-day-old) guinea pigs on standard (0.76% Pi), low (<0.1% Pi), and high (1.3% Pi) Pi diets. (From Neiberger et al. [24])

collaboration with Neiberger and Barac-Nieto [24], we studied the transport of Pi in luminal brush border membrane vesicles (BBMV) obtained from kidneys of newborn and adult animals (Fig. 3). At both ages, most transport of Pi into the vesicles was found to be Na^+ gradient dependent. The uptake was concentrative, i.e., the intravesicular Pi concentration exceeded the equilibrium concentration, as long as an inwardly directed Na^+ gradient subsisted across the brush border membrane. As the gradient dissipated, the vesicular Pi content diminished to reach equilibrium. The initial rate of Na^+-dependent Pi uptake was linear with time and was much higher in vesicles obtained from newborn than from adult animals. Kinetic analysis of the initial transport rates revealed that the V_{max} of Na^+-Pi was substantially higher in BBMV from newborns (650 pmol/s per mg protein) than those of adults (144 pmol/s per mg protein) (Fig. 4). The K_m, a measure of the apparent affinity of the cotransporter for Pi, did not differ with age. Thus, the capacity for Na^+-Pi cotransport across the luminal brush border membrane of renal proximal tubule cells is more than fourfold higher in the newborn than in the adult.

We also studied the consequences of the high Pi reabsorptive capacity on the ability of the newborn to regulate Pi in the face of changes in dietary supply of Pi [24]. Supplementing the diet with Pi for 3 days decreased by about 50% the V_{max} for Na^+-Pi cotransport in renal BBMV from adult animals (Fig. 5). The V_{max} also decreased in the membrane vesicles from the newborn, but the relative change was only about 25%. A low dietary Pi resulted in more than doubling of the Na^+-Pi cotransport capacity in renal BBMV of the adult (from 144 to 318 pmol/mg per s), but there was no significant change in the V_{max} of the Na^+-Pi cotransport system in the newborn. The serum Pi concentration varied in proportion to the Pi supply in the newborn, but remained constant in the adult. The results demonstrate that in the newborn the Na^+-Pi cotransport system is characterized by a high

transport capacity but a low adaptability to changes in dietary Pi. The tendency to retain Pi and the more limited ability to adapt to changes in Pi intake may explain the hyperphosphatemia observed in newborns fed a diet of cows' milk, which is rich in Pi [7].

Cellular mechanisms involved in the enhanced Pi reabsorption associated with growth: role of intracellular Pi concentration

The higher V_{max} of Na^+-Pi cotransport observed in microvilli of the newborn compared with the adult cannot be accounted for by age-related differences in plasma concentration of Pi. Moreover, the high capacity of the developing kidney for Pi reabsorption persists even when the organ is isolated and perfused in vitro [10], and thus is independent of the continuous presence (or absence) of extracellular factors known to modulate renal Pi transport in vivo. There is, on the other hand, evidence that renal Pi reabsorption is affected by total body stores of Pi [25]. Is it possible that the high Pi demand associated with growth may, similar to a reduction in dietary Pi supply, result in a low level of intracellular Pi, and that this low level is responsible for initiating and maintaining a high renal Pi cotransport capacity? To test this hypothesis, we used nuclear magnetic resonance (NMR) spectroscopy and chemical methods to determine the effect of age and Pi intake on intracellular Pi in the perfused kidney. We related these results to measurements of V_{max} for Na^+-Pi cotransport in renal microvilli derived from animals of similar age fed the same diets [26]. In animals fed a normal diet intracellular Pi was twice as high (1.85±0.23 vs. 0.90±0.02 mM) while fractional reabsorption of Pi was lower (0.70 vs. 0.90) in >4- than in <1-week-old animals. Diet-induced changes in intracellular Pi were associated with reciprocal changes in V_{max} of similar absolute magnitude in immature and mature animals. However, at any given intracellular Pi concentration, V_{max} was substantially higher in microvilli prepared from kidneys of newborn than of older animals (Fig. 6). The findings indicate that changes in intracellular Pi related to age or diet may be a cause, but are not a consequence, of changes in abundance or maximal mobility of Na^+-Pi cotransporters. Data also indicate that factors in addition to low intracellular Pi contribute to the high Na^+-Pi cotransport capacity observed in microvilli of growing animals.

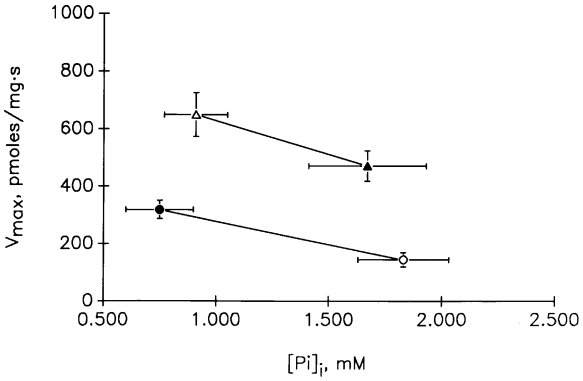

Fig. 6 Relationship between intracellular Pi ($[Pi]_i$) in kidney and V_{max} in renal microvilli of newborn guinea pigs fed standard (*open triangle*) or high (*solid triangle*) Pi diets and mature guinea pigs fed standard (*open circle*) or low (*solid circle*) Pi diets. (From Barac-Nieto et al. [26])

The role of growth hormone

A role for growth hormone (GH) in the regulation of Pi reabsorption was predicated on the basis of clinical observations of patients with acromegaly [27]. Studies in proximal tubule brush border vesicles revealed the direct stimulatory effect of GH on Na^+-Pi uptake [28]. Finally, administration to young rats of an antagonist to GH-releasing factor resulted in a substantial decrease in Pi reabsorption and growth [29]. In order to address this issue, we measured net tubular transport of Pi (TPi) and NMR-visible Pi in 8-week-old genetically GH-deficient dwarf rats, and in dwarf rats treated with GH (100 µg/day for 3 days). In addition, we evaluated the effect of insulin-like growth factor-I (IGF1), the mediator of GH, on ^{32}P uptake and intracellular Pi in OK cells incubated for 12 h at various medium Pi concentrations [30]. As expected, the fractional reabsorption of Pi was low in the dwarf rats. Administration of GH resulted in an almost 80% increase in the filtered load of Pi, due mainly to an increase in GFR. This was associated with a nearly identical rise in the absolute reabsorption of Pi and hence with little change in the excretion of Pi. However, the intracellular concentration of Pi, which was very low in the GH-deficient rats, changed very little, if at all, when GH was administered. A similar pattern was observed in proximal tubule-like cells grown in culture [31]. Exposure of OK cells to IGF1 (10^{-8} M) resulted in a 25% increase in Pi uptake, which was associated with a small increase, rather than a decrease, in NMR-visible Pi. When OK cells were deprived of Pi for 12 h, there was a large decrease in intracellular Pi that was associated with the expected increase (37%) in Pi uptake. Addition of IGF1 to the media of the Pi-deprived cells resulted in a further increase in Pi uptake of a magnitude similar to that observed in cells exposed to 1 mM Pi. This latter change in Pi occurred in the absence of additional changes in intracellular Pi. The results of these experiments confirmed our assumption that the high rates of renal reabsorption of Pi uptake prevailing during development are the result of at least two independent but complementary factors: high circulating levels of GH and low intracellular levels of Pi.

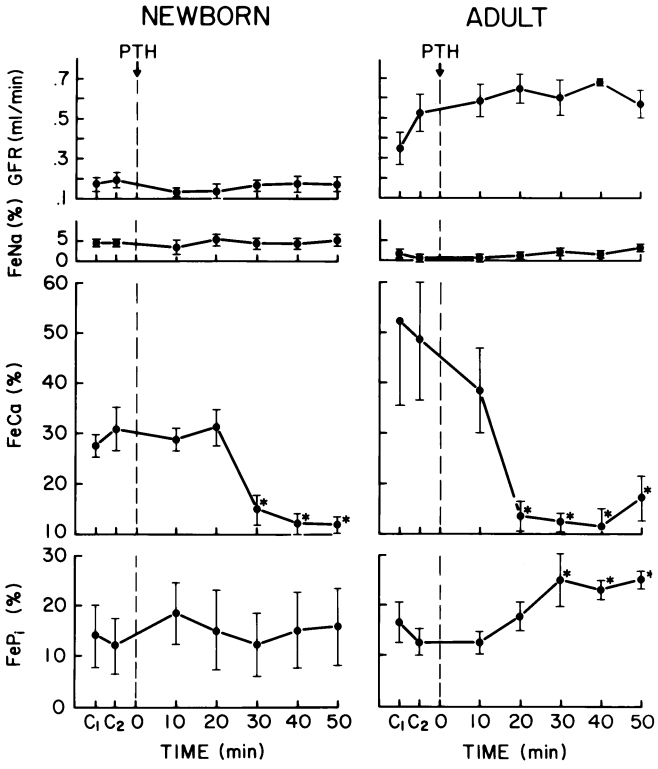

Fig. 7 The effect of parathyroid hormone (*PTH*) on fractional excretion of Na^+, Ca^{2+}, and Pi in isolated perfused kidneys from newborn and adult guinea pigs. *P<0.01 compared with control values. (From Johnson and Spitzer [10])

The effect of parathyroid hormone on renal Pi transport during development

In the adult, parathyroid hormone (PTH) decreases the excretion of Pi by inhibiting brush border membrane Na^+-Pi cotransport [28, 32]. This may involve an endocytic retrieval of NaPi-2 cotransporters from the apical membrane [33, 34, 35], possibly mediated by protein kinases A and/or C [36]. The response of the kidney to PTH is blunted during early postnatal life. Infusion of PTH into newborn babies resulted in a minimal depression in the tubular reabsorption of Pi [37]. In isolated kidney of newborn guinea pigs, addition of PTH to the perfusion fluid caused a marked increase in tubular calcium (Ca^{2+}) reabsorption and in urinary excretion of cAMP, but affected little the excretion of Pi [10] (Fig. 7). The result is retention of both Ca^{2+} and Pi, as required for growth. The blunted effect of PTH on Pi transport in the newborn may be due to a lower cytosolic phospholipase A_2 activity [38].

Molecular mechanisms of Pi transport

Work performed during the last decade by Murer, Biber and their collaborators led to the identification of multiple Na^+-Pi cotransporters in renal tubules. The cRNA de-

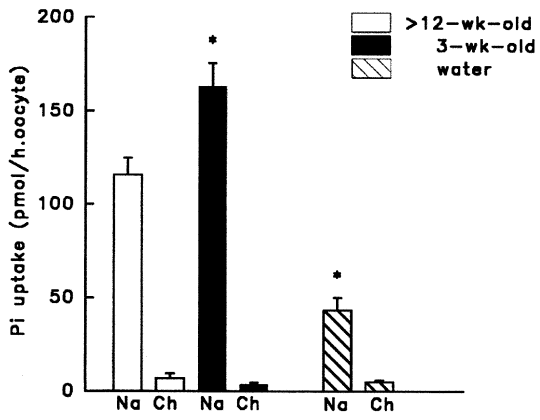

Fig. 8 ^{32}Pi uptake (dpm/h) by oocytes injected with water (50 nl, *crossed hatched bars*) or with renal cortical polyadenylated RNA (0.5 ng/nl, 50 nl) derived from >12-week-old (*open bars*) or 3-week-old (*solid bars*) rats. Three days after the injections, the oocytes were exposed to 0.5 mM ^{32}Pi (5 μCi/ml) in an isosmotic NaCl solution or, where indicated, in a solution containing choline (*Ch*) instead of Na^+. *P<0.05 vs. controls injected with polyA RNA from adult rats. Data are expressed as mean±SE (n=3)

rived from the first cotransporter cloned (NaPi-1) induced expression of Na^+-dependent Pi transport in oocytes [39]. The abundance of this mRNA in renal cortex did not change with Pi depletion. Another transporter (NaPi-2) was cloned from a rat renal cortex cDNA library [40]. NaPi-2 has only 20% homology with NaPi-1, but is 90% homologous to NaPi-3, cloned from a human renal cortical cDNA library [41]. Both are modulated by changes in Pi intake. Type II transporters highly homologous to NaPi-2 and NaPi-3 were cloned from rabbit renal cortex (NaPi-6) [42] and from OK cells (NaPi-4) [43]. These discoveries prompted us to investigate the molecular mechanism responsible for the high rate of renal Pi transport present during growth. For obvious reasons, we focused on the NaPi type II transporter, found in the kidney of the rat.

Effect of age on polyA RNA expression and ^{32}Pi uptake in oocytes

Confirming our finding in guinea pigs [24], the rate of Na^+-dependent Pi uptake, at 0.5 mM Pi, a concentration 5 times higher than the K_m reported for NaPi type II transporters, was threefold higher in BBMV of younger than of older rats. In order to ascertain that the high rates of Pi transport observed in the young are genetically controlled, we measured the effects of renal cortical polyA RNA derived from 3- and >12-week-old rats on Na^+-Pi cotransport in oocytes [44]. Uptake was 50% higher in oocytes injected with polyA RNA derived from 3- than from >12-week-old rats (Fig. 8). Furthermore, the oocytes injected with polyA RNA from adult kidney exhibited >3 times higher Na^+-dependent ^{32}Pi uptake than oocytes injected with water. The age-related difference in functional expression could be explained either

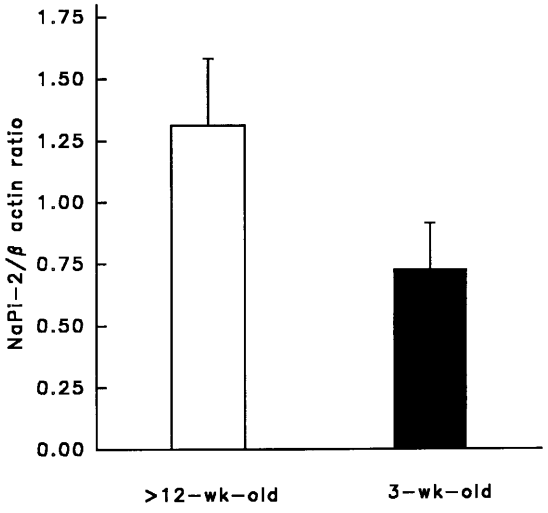

Fig. 9 Densitometric analysis of northern blots of renal cortical RNA, obtained from 12- and 3-week-old rats, hybridized with a ^{32}P-NaPi-2 probe (n=15)

by the presence in the polyA RNA of the young of a NaPi-2 regulatory protein, or of an abundant mRNA encoding for a Na^+-dependent Pi transporter.

Abundance of NaPi-2 mRNA in renal cortex of newborn and adult rats

Because of the similarities between adaptation of the kidney to a high Pi demand (growth) and that to low Pi supply, we proceeded to measure the levels of NaPi-2 mRNA transcripts in kidney cortex of 3-week-old and >12-week-old rats. RNA obtained from renal cortex of adult rats showed a strong hybridization with the NaPi-2 probe, at 2.7 kilobases. The hybridization signals detected with renal cortical RNA derived from 3-week-old rats were of similar or lower intensity than those observed with RNA from adult rats, even when normalized for the amount of RNA loaded, as assessed by reprobing the blots with β-actin cDNA. Densitometric measurements of the northern blots confirmed the visual assessment (Fig. 9).

Abundance of NaPi-2-like protein in renal cortex of young and adult rats

The apparent discrepancy between the NaPi-2 mRNA levels and the high rates of Na^+-Pi cotransport observed in renal BBMV of the young prompted us to determine the expression of NaPi-2 protein in BBMV and renal cortex of 3-week-old animals, and compare the results with those observed in the >12-week-old rats. The western blots (Fig. 10) illustrate the expression of NaPi-2 and γ-glutamyl transpeptidase (γ-GT) proteins in renal brush border membrane of 3- and >12-week-old rats. Each lane represents 20 μg brush border membrane proteins from a

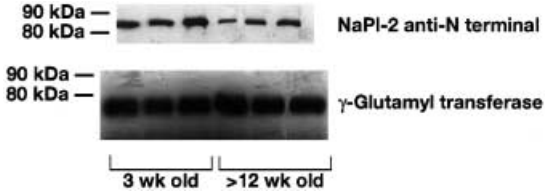

Fig. 10 Western blot of NaPi-2 and γ-glutamyl-transferase (γ-GT) in brush border membrane of young and adult animals. Note the age-dependent difference in the expression of NaPi-2 and the similar expression of γ-GT protein at the two ages

different group of 3-week-old rats or from an individual >12-week-old rat. A single band, between 80–90 kilodaltons (kDa), that reacts strongly with NaPi-2 antiserum, is 2–3 times more abundant in 3- than in >12-week-old animals. The 75- to 80-kDa protein recognized by a γ-GT-specific antibody in the same brush border membrane preparations does not change with age. Using immunohistochemistry and in situ hybridization Traebert et al. [45] demonstrated that the appearance of the NaPi-2 protein in the kidney of the rat coincides with the development of the brush border. Its overall abundance peaks around 13 days of extrauterine life and decreases to adult values by 6 weeks of age.

The results of the experiments described so far are compatible with the existence of two mechanisms of action. Indicative of transcriptional regulation, and thus of an mRNA that encodes for a growth-specific NaPi protein, is the discrepancy between the rates of Na^+-Pi cotransport induced in oocytes by polyA RNA from the newborn and those induced by an equal amount of polyA RNA from the adult. These observations suggest that post-transcriptional regulation (e.g., regulatory protein) of NaPi-2 is responsible for the discrepancy observed in the newborn between the levels of NaPi-2 mRNA and those of NaPi-2-like protein. Alternatively, the discrepancy between the mRNA expression and NaPi-2-like protein observed in the young may be due to the relative lack of specificity of the antibody used to detect the protein. That the latter may be the case is suggested by the fact that the antibody is directed against a short sequence (12 amino acids) of the N-terminal segment of the NaPi-2 protein. This region of the NaPi-2 protein is encoded by a mRNA region rich in degenerate codons, and hence, the antibody may detect other NaPi transporters. It should be emphasized that the two regulatory mechanisms may co-exist.

Effect of NaPi-2-depleted polyA RNA on the induction of Na^+-Pi cotransport in oocytes

In order to explore this possibility, Silverstein et al. [44, 46] hybridized an oligodeoxynucleotide capable of annealing (antisense) to the open reading frame [1,004–1,019 base pairs (bp)] of the NaPi-2 mRNA transcript to polyA RNA derived from 3- and >12-week-old rats, or to NaPi-2 cRNA, and assessed the effect of the

hybrids on induction of Na^+-Pi cotransport in oocytes. In the controls, the sense 16-mer was substituted for the antisense 16-mer in the annealing reaction. The hybridized polyA RNA samples were also digested with RNase H and used for reverse transcription polymerase chain reaction (RT-PCR) using a primer pair (for positions 943–958 and 1,018–1,005 of NaPi-2 cDNA) to amplify a 75-bp NaPi-2-specific product. PolyA RNA of 3- and >12-week-old rats exposed to the sense primer generated the expected 75-bp specific PCR product. Annealing the polyA RNAs to the antisense oligomer, followed by RNase H digestion of the hybrids, annihilated their ability to serve as templates for the generation of the NaPi-2-specific product. However, annealing with the antisense abolished only the ability of polyA RNA of adult (and that of the cRNA), *but not that of the young* rats, to induce Na^+-Pi cotransport in oocytes. These results demonstrate that the induction of Na^+-Pi cotransport by renal cortex polyA$^+$ RNA of young rats is largely independent of the presence of NaPi-2 mRNA.

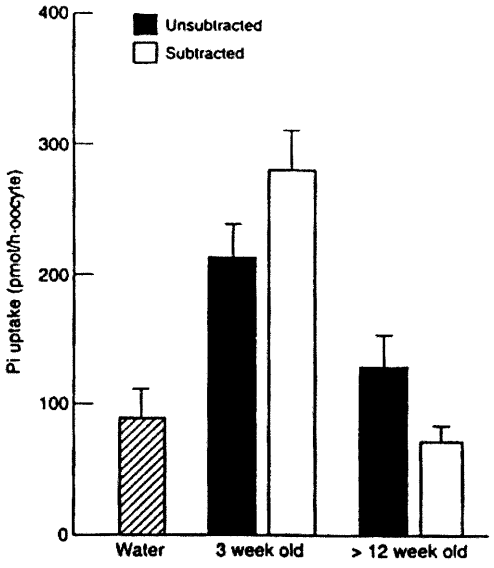

Fig. 11 Pi uptake in oocytes injected with renal cortical polyA RNA from 3-week-old rats before and after three cycles of hybridization to cDNA generated using oligo(dT) primers and polyA RNA of mature rats. Data are mean±SE of 9 experiments using 5–10 oocytes for each experimental condition. (From Silverstein et al. [46])

Subtractive hybridization of renal cortical polyA RNA

Further evidence in favor of the existence of a growth-specific NaPi-2 isoform was obtained from hybrid depletion experiments performed by Silverstein et al. [46]. The basic postulate of the subtraction procedure is that the polyA RNA from the experimental animal hybridizes with cDNA from the control animal. In our studies, polyA RNA (5 mg) derived from 3-week-old rats was hybridized with cDNA generated from 5 mg of renal cortex polyA RNA of adult rats. In order to test the effectiveness of the subtractive hybridization, renal cortex polyA RNA from >12-week-old rats was hybridized with cDNA generated from rats of the same age [47]. One subtraction cycle was sufficient to remove the NaPi-2 transcripts and abolish the ability of this polyA RNA to induce Pi uptake in oocytes. To assess further the effectiveness of the subtraction, β-actin- and NaPi-2-specific primers were used to check for amplification of any residual β-actin and NaPi-2 mRNA. The β-actin mRNA, a very abundant transcript, was still detectable, albeit in small quantities, after two hybridization passages, but generally absent after the third. NaPi-2 mRNA, an uncommon transcript, was consistently absent after three passages. Because NaPi-2-specific RT-PCR product may have escaped detection due to lack of sensitivity of the ethidium bromide staining, we blotted the gels containing the RT-PCR product onto nylon membranes and carried out Southern hybridization using a ^{32}P-CTP labeled full-length cDNA NaPi-2 probe. The absence of residual NaPi-2 transcripts was confirmed. However, Na^+-dependent Pi uptake was similar in oocytes injected with polyA RNA harvested after the third subtractive passage (277±31 pmol/oocyte per hour) and in those injected with polyA prepared prior to subtraction (212±26 pmol/oocyte per hour) (P>0.3) (Fig. 11). As a control, adult polyA RNA was exposed to subtraction with adult renal cortical cDNA. The remaining transcripts were injected into oocytes. Prior to subtraction, Na^+-dependent Pi uptake into oocytes injected with adult renal cortical polyA RNA was 128±24 pmol/oocyte per hour. After subtraction, Na^+-dependent Pi uptake into oocytes (89±24 pmol/oocyte per hour) was reduced to barely above that demonstrated by water-injected oocytes. The results confirm that polyA RNA from young rats, depleted of NaPi-2 mRNA by subtractive hybridization, preserved its ability to encode for Na^+-Pi transport in oocytes. This conclusion is compatible with the observation that young Npt2 (–/–) knockout mice, lacking Npt2 mRNA and protein, retain the capacity to reabsorb phosphate at a rate that cannot be explained by the presence of type I and III Pi transporters. These animals were born small and were hypophosphatemic, but they grew at rates similar to their normal counterparts [48, 49].

In order to determine whether the transcript responsible for the high rate of Pi uptake induced by polyA RNA was present at all ages, but was more abundant in the young, we hybridized polyA RNA of growing rats to cDNA derived from a fivefold excess (25 mg) of renal cortical polyA RNA from >12-week-old rats. Exposure to excess adult cDNA did not reduce the ability of subtracted polyA RNA from growing animals to induce Pi uptake [47]. This provided additional evidence that renal cortical polyA RNA of the young contains a NaPi mRNA transcript that is absent in the mature rat.

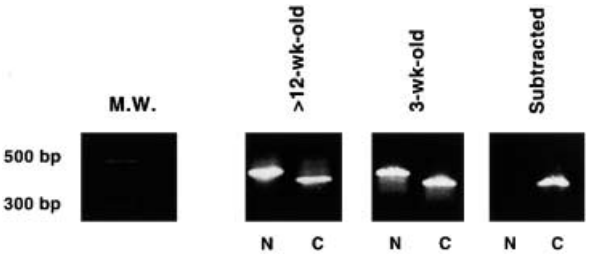

Fig. 12 Reverse transcription-polymerase chain reaction amplification products generated by using as a template polyA RNA isolated from renal cortex of >12- and 3-week-old rats, or polyA of 3-week-old rats hybridized with cDNA prepared from renal cortex polyA of >12-week-old rats. The primers were specific for NaPi-2 (*N*) or for a region conserved in all type II (modulated) NaPi cotransporters (*C*). (From Silverstein et al. [46])

Detection of a conserved region of a NaPi type II transcript in polyA RNA of NaPi-2 depleted kidney of the young

The results described above could be explained either by the presence of a growth-specific mRNA or of a regulatory protein that stimulated the oocyte constitutive NaPi transporter. We reasoned that any NaPi like transcript specific to the kidney of growing rats should share sequences with most type II NaPi transporters. To test this hypothesis, the subtracted (NaPi-2-depleted) mRNA transcripts were used for: (1) RT-PCR with primers for a region of the NaPi-2 sequence (1,276–1,657 bp) highly conserved in Na^+-Pi type II (Pi modulated) cotransporters; or (2) RT-PCR with NaPi-2-specific primers (1,544–1,966 bp). RT-PCR of polyA from the young subjected to subtraction, using primers to amplify the highly conserved region, resulted in an abundant signal of the expected size (379 bp), while RT-PCR using primers specific for NaPi-2 cDNA (423 bp) did not generate the expected product (Fig. 12). These results demonstrate that a polyA RNA transcript from the renal cortex of growing rats contains a nucleotide sequence identical to that present in all known type II Na^+-Pi cotransporters. This likely represents part of the message for a NaPi transporter isoform unique to the kidney cortex of growing rats.

Conclusions

The data presented herein illustrate the mechanism by which the "immature" kidney achieves the high rates of Pi reabsorption required for the maintenance of a positive external balance. This is the first time that the existence of a putative growth-specific transporter has been documented. It is likely that this is not the only one. Of further interest is the fact that this finding adds to the diversity of mechanisms by which the kidney adapts to the needs of the growing organism. The high rates of Na^+ reabsorption observed during growth are accounted for by enhanced transport at the level of distal nephron segments [50, 51, 52, 53], stimulated by the high levels of aldosterone prevailing at this age [54, 55]. However, the excretion of potassium, which in the adult would be increased under these circumstances, is low in the infant do to the paucity of potassium channels in the collecting ducts [56, 57, 58]. Finally, the renal retention of Pi is mediated, at least in part, by a growth-specific, Na^+-dependent Pi transporter. All these processes, and perhaps others, although profoundly different in nature, converge towards the preservation of a chemical environment that allows cells to grow, multiply, and differentiate.

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