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COMMENTARY

CURRENT CONCEPTS OF REGULATION OF PHOSPHATE TRANSPORT IN RENAL PROXIMAL TUBULES*

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In the kidney, the inorganic phosphate (P_i†) which is filtered at the glomerulus is reabsorbed primarily in the proximal tubule [1, 2]. The initial step in this process is Na^+-gradient-dependent transport of P_i across the brush border membrane (BBM) which faces the tubular lumen. Reabsorption of P_i in this nephron segment is altered by various drugs and hormones and by the dietary phosphorus intake [1, 2]. In most of these situations, the change in P_i reabsorption is accompanied by a specific change in the capacity of the Na^+-dependent P_i transport system localized in the BBM [3]. In this commentary, we have attempted to illustrate common trends among published data and to present some fresh concepts. We suggest that the numerous factors which regulate P_i transport across the renal BBM can be hypothetically grouped into two general classes: (a) those which produce a rapid response (within minutes and less than 1–2 hr), and (b) those which produce a measurable effect only after 12–24 hr or longer. Rather than considering each of these factors in detail, our main purpose is to provide some insights into the different underlying mechanisms which may be involved in these rapid and long-term responses. The biochemical composition of the renal BBM transport system for P_i is virtually unknown. However, from indirect observations, we can assume that a specific structure exists in BBM, probably an intrinsic protein or glycoprotein, which constitutes a specific route for Na^+-P_i cotransport. Such a hypothetical structure will be referred to as the "Na^+-P_i cotransporter" (Na^+-P_i-COT).

RAPID RESPONSES OF RENAL BBM TRANSPORT FOR P_i

General comments

Rapid changes in the rate of P_i transport across the renal BBM could be achieved by either (a) *in situ* modifications of existing Na^+-P_i-COT or (b) a shift in the distribution of Na^+P_i-COT between the BBM and cytoplasmic depots. Neither of these processes would require *de novo* synthesis of glycoproteins and proteins, which is in agreement with the observations that inhibitors of translation and transcription do not block the response [4–6]. A third possibility may involve a combination of these processes.

In situ modification of Na^+-P_i-COT already present in the BBM represents the rather traditional view of the mechanism of action of various, regulatory factors, mainly hormones (Fig. 1). Possible mechanisms which could be involved in regulating *in situ* the Na^+-P_i-COT are cyclic AMP-dependent phosphorylation [3, 7, 8], or NAD-dependent ADP-ribosylation [8, 9], or Schiff base formation [9, 10]. Both phosphorylation and ADP-ribosylation of isolated BBM vesicles *in vitro* were associated with specific inhibition of P_i transport [7, 8]. Thus, there is evidence to suggest that P_i transport can be regulated by modification of Na^+-P_i-COT already inserted in the BBM. It should be stressed that, in other rather careful studies, ADP ribosylation and protein phosphorylations were not found associated with changes in BBM transport of P_i [11, 12]. The importance of these mechanisms under *in vivo* conditions remains to be determined.

Shuttling the Na^+-P_i-COT between the functioning pool in BBM and cytoplasmic inactive depot vesicles, the phenomenon of membrane recycling [13–16], is an alternative or additional mechanism which may play a role in rapid regulation of BBM transport of P_i (Fig. 1). In contrast to the relatively slow turnover of membrane phospholipids and glycoproteins, recycling of membrane internalized by endocytosis is extremely rapid [11, 12]. It occurs in the proximal tubule cell [11] and may be a potential mechanism for changing the number of Na^+-P_i-COT within the BBM. The total number of Na^+-P_i-COT per one cell would not change. Since an intact cytoskeleton, namely microtubules, is needed for exocytosis [16], it is interesting to note that colchicine, which disrupts microtubules, interferes with the regulation of renal P_i transport by parathyroid hormone (PTH) [17] and BBM transport of P_i as well [18]. Phosphorylation or ADP-ribosylation *in vivo* may induce changes in Na^+-P_i-COT recycling, leading to changes either in the *rate of* insertion of Na^+-P_i-COT into the BBM or in the rate of retrieval back to the cytoplasm, located in clathrin-coated vesicles. This proposal requires that BBM domains [19] containing the Na^+-P_i-COT are under specific control and can be recycled when necessary. Precedents for a recycling mechanism of specific domains involving specific transport systems come

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† Abbreviations: P_i, inorganic phosphate; Na^+-P_i-COT, Na^+-P_i cotransporter(s); BBM, brush border membrane; PTH, parathyroid hormone; and T_3, triiodothyronine.

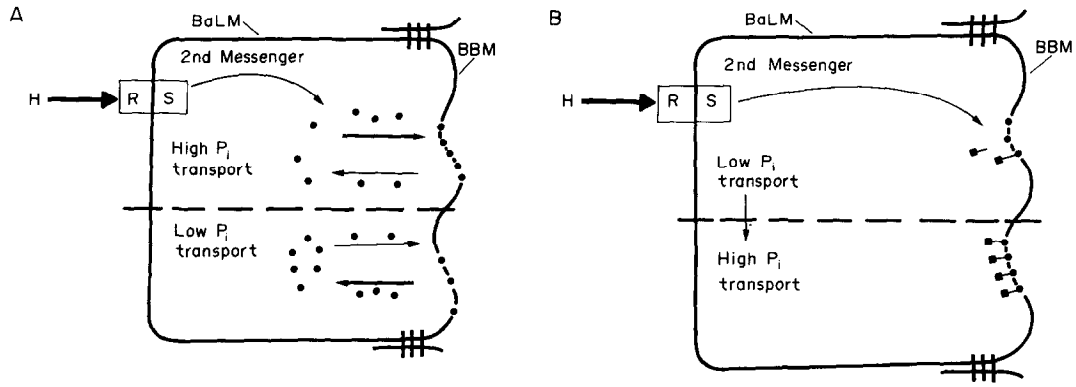

Fig. 1. Scheme of regulation of P_i transport by "rapid" stimuli. A hormone (H) binds to a receptor (R) and generates a signal (S)—2nd messenger—which either: (A) increases exocytotic insertion of Na^+-P_i-COT (●) from a cytoplasmic pool of clathrin-coated vesicles into the BBM, or (B) causes *in situ* covalent modification of Na^+-P_i-COT within the BBM by a mechanism, such as phosphorylation. Key: (●) inactive Na^+-P_i-COT, and (●—■) modified active Na^+-P_i-COT.

from studies of H^+ pumps in proximal tubule [20] and vasopressin-induced water channels [21] and glucose transport systems in adipocytes [22]. In a recycling mechanism, "2nd messenger" would operate *within cytoplasm*, rather than at BBM.

A third theoretical possibility to be considered is that Na^+-P_i-COT may be modified in the cytoplasmic pool prior to insertion in the BBM. This seems the least likely of these proposals, and we are not aware of experimental data to support it.

In situ covalent modification of the Na^+-P_i-COT and cycling of the Na^+-P_i-COT have several features in common (Fig. 1). In both cases, *de novo* synthesis of Na^+-P_i-COT need not be changed and the total number of Na^+-P_i-COT per cell of proximal tubule remains the same. This is in good agreement with the observations that blockers of polypeptide synthesis do not interfere with the rapid stimuli. Second, it seems common for rapid stimuli, which are mainly hormones, that the changes are signaled intracellularly by the "2nd messengers". Usually hormone–receptor interaction occurs at the basal lateral membrane where the intracellular "2nd messenger" regulatory pathway initiates. The end point of a "2nd messenger" regulatory pathway either (a) causes covalent *in situ* BBM modification of Na^+-P_i-COT, or (b) within cytoplasm causes a change in the rate of BBM-cytoplasm recycling of Na^+-P_i-COT. Several "2nd messenger" pathways were described recently as possible mechanisms in proximal tubules and include classic cAMP protein phosphorylation, phosphatidylinositol cycle calcium-protein C kinase, and NAD actions via enzymatic (ribosyl transferase) or non-enzymatic reactions. These distinct types of intracellular regulatory pathways need not be mutually exclusive. One type of "2nd messenger" mechanism may be specific for a given rapid regulatory hormone (for example, PTH), or a given hormone may trigger more than one mechanism, but each mechanism may regulate Na^+-P_i-COT in a different subsegment of the proximal tubule, such as the convoluted segment and the late pars recta. Also, the pathways may operate in parallel as a "redundant" control to assure the final effect on Na^+-P_i-COT.

An intracellular pathway which regulates events in the cytoplasm would seem to be a much more economical mechanism than one which requires transfer of the "message" across the whole cell to act on the Na^+-P_i-COT *in situ* within the BBM.

Specific factors

Factors which induce a rapid response in BBM phosphate transport include mainly specific hormones, such as PTH [23, 24], growth hormone [24], calcitonin [25], insulin [26], and atrial natriuretic factor [27], nicotinamide [3, 9, 28], and acute changes in P_i intake [29, 30] or in perhaps the plasma CO_2 concentration [31, 32] (Table 1).

The effects of PTH and acute P_i deprivation persist in animals treated with actinomycin D [4, 5], which is the basis for suggesting that rapid stimuli do not depend on *de novo* protein synthesis. Another com-

Table 1. Summary of factors producing rapid changes in BBM P_i transport

Factors	Principal characteristics of induced response
Parathyroid hormone Growth hormone Calcitonin Acute phosphate loading Acute phosphate deprivation Plasma CO_2 Nicotinamide Insulin Atrial natriuretic factor (ANF)	Change in BBM P_i transport occurs within minutes, less than 1–2 hr, and is typically a V_{max} effect. It is not prevented by inhibitors of protein synthesis.

mon property appears to be that these factors induce a change in the V_{max} rather than the K_m for P_i [24, 30]. Both *in situ* modification and membrane recycling mechanisms are compatible with a change primarily in V_{max}.

The intracellular mechanism of action of these "rapid" factors remains to be established but the results of recent intensive investigations offer some clues. Perhaps the most widely studied factor has been PTH. A possible sequence of events leading to *in situ* modification of BBM phosphate transporters has been summarized recently [7, 8]. Binding of this hormone to its receptor in the basolateral membrane stimulates the production of cyclic AMP as an intracellular "2nd messenger", activates a cyclic AMP-dependent protein kinase in the BBM, which phosphorylates specific BBM proteins, and leads to inhibition of P_i transport. This scheme is supported by observations that cyclic AMP-dependent phosphorylation of isolated BBM vesicles is accompanied by specific inhibition of Na^+-gradient-dependent P_i transport, and that inhibition of phosphate transport is relieved by subsequent dephosphorylation [7, 8].

Alternative or additional "2nd messenger" mechanisms which may be part of the regulation by PTH include changes in intracellular free calcium ions [33] and the phosphatidylinositol-protein C kinase system [34–37].

Another proposed mechanism by which PTH may induce *in situ* modification of Na^+-P_i-COT in the BBM is via a change in the intracellular system involving gluconeogenesis and NAD. Stimulation of the rate of gluconeogenesis in the proximal tubule produces an increase in the NAD/NADH ratio leading to an increase in the amount of free cytosolic NAD which is available for ADP-ribosylation of BBM proteins [3, 9] or for other changes within cytoplasm. This proposal derives support from studies both *in vivo* and *in vitro*. A phosphaturic dose of PTH stimulates renal gluconeogenesis [38] and increases the NAD/NADH ratio in renal cortex in the rat [39]. Administration of nicotinamide, which directly increases the NAD level of renal cortex, is accompanied by marked phosphaturia and inhibition of BBM transport of P_i [3, 9]. Trapping NAD within isolated BBM vesicles leads to ADP-ribosylation of BBM proteins and specific inhibition of P_i transport [8], and preliminary evidence indicates that PTH may stimulate the ADP-ribosylation mechanism [40].

These ideas have stimulated a great deal of controversy and criticism. There have been reports that changes in the rate of renal gluconeogenesis are not causally related to changes in phosphate transport [41, 42], and others have raised questions about the *in vitro* studies on phosphorylation and ribosylation of isolated BBM vesicles [11, 12, 43]. The proposed role for NAD [3, 9] has been also criticized on the basis of the results of *in vitro* studies [44, 45], but this conclusion may be premature in the light of subsequent findings [46–48].

It is worth mentioning at this point that the concept of internephron and intranephron (axial) heterogeneity of P_i transport by proximal tubules [2, 49] is reflected in studies with isolated BBM, especially with regard to the locus of action of PTH and calcitonin. These hormones stimulate or inhibit P_i transport to a greater degree in BBM vesicles from juxtamedullary cortex–outer medullary tissue [25] even though the net rate of P_i transport is highest in BBM vesicles derived from superficial cortex [50].

Loading thyroparathyroidectomized (TPTX) rats with P_i produces a rapid rise in plasma P_i and there is a decrease in BBM P_i transport within 40 min [29]. Feeding low P_i diet leads to a fall in plasma P_i and BBM transport of P_i is increased within 4 hr [30]. These observations may suggest that acute changes in plasma P_i might be the signal which initiates the changes in BBM transport. Changes in intracellular P_i, specifically the cytosolic compartment, may also be an important factor since depletion of cytosolic phosphate may impair mitochondrial respiration and ATP production [51, 52]. Indeed, preliminary data from NMR studies indicate that the phosphaturia induced by acute P_i loading is accompanied by a change in cytosolic free P_i [53]. These possibilities were investigated recently by the use of acute fructose loading to "trap" intracellular P_i in the form of phosphorylated intermediates of glycolysis [54, 55]. This procedure decreased the levels of P_i in both plasma and renal cortical tissue and decreased tissue ATP within 1 hr, but there were no changes in BBM P_i transport [47, 48]. Thus, there is little tangible support for the notion that acute changes in plasma P_i or intracellular P_i may regulate BBM transport of P_i.

As yet there is no direct evidence to suggest that Na^+-P_i-COT recycling plays a central role in the response of the BBM to rapidly acting factors. However, if it does, then the presence of cytoplasmic factors may be essential. The lack of these factors in isolated washed BBM vesicles may explain why the putative inhibitory effects of phosphorylation or ribosylation on P_i transport were not found in some very carefully conducted studies [11, 12, 43]. Since many steps in gluconeogenesis occur in the cytoplasm (namely in rat nephron), this process could be one of the cytoplasm factors influencing the recycling process. Correlative comparisons show that changes in gluconeogenesis often accompany changes in renal P_i transport *in vivo* [9]. While the mechanism remains to be defined, the changes in gluconeogenesis, NAD content or NAD/NADH ratio in cytoplasm may influence specific recycling of Na^+-P_i-COT.

LONG-TERM ("ADAPTIVE") RESPONSES

General comments

These responses are prevented by inhibitors of translation and transcription [56–59] which suggests that *de novo* mRNA and polypeptide synthesis and membrane biogenesis are an integral part of the cellular mechanism. Assuming that the Na^+-P_i-COT is a protein, probably a glycoprotein, then biogenesis will involve both co-translational and post-translational processing in the rough endoplasmic reticulum and Golgi followed by movement to, and insertion in, the BBM (Fig. 2). The sequence of events at each of these steps is likely to be specific for the Na^+-P_i-COT, compared to other BBM proteins,

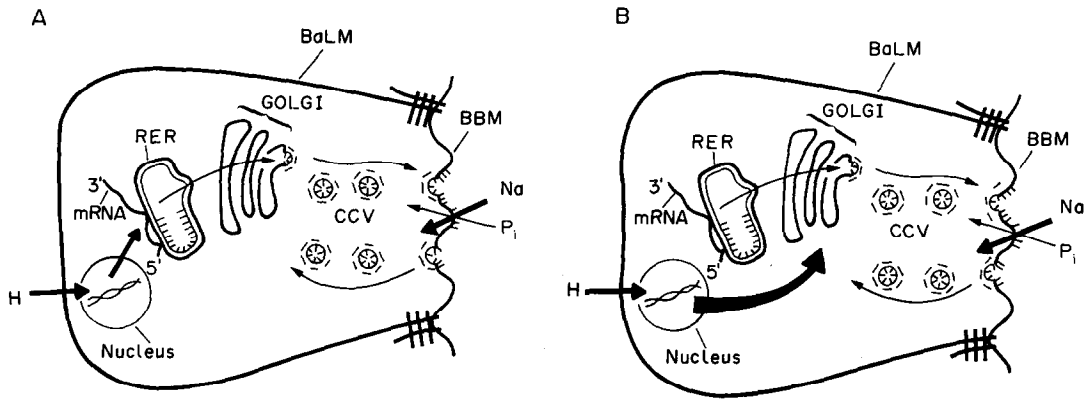

Fig. 2. Scheme of regulation of Na^+-P_i-COT biogenesis by "adaptive" stimuli. A hormone (H) enters the nucleus and either: (A) stimulates synthesis of mRNA coding nascent Na^+-P_i-COT in rough endoplasmic reticulum (RER), or (B) stimulates synthesis of various mRNA coding enzymes of post-translational processing of the Na^+-P_i-COT; after processing, the Na^+-P_i-COT is transferred in clathrin-coated vesicles (CCV) into BBM. After uncoating and fusion, the Na^+-P_i-COT is inserted into BBM and becomes available for P_i transport. Na^+-P_i-COT can be reclaimed by endocytosis back into the cytoplasmic depot pool.

transport systems and enzymes. When biogenesis of the Na^+-P_i-COT is altered during adaptive responses, a change in the number of Na^+-P_i-COT (V_{max} effect) per one cell, in the properties of the transporters (K_m effect), or both might occur.

If a long-term adaptive stimulus, e.g. triiodothyronine (T_3) or glucocorticoid, acts to change BBM transport of P_i via the "nuclear mechanism", the common feature in activation of *de novo* mRNA synthesis, then two possibilities can be envisioned: (A) Stimulus simply increases the rate of synthesis of nascent Na^+-P_i-COT in rough endoplasmic reticulum. The total number of Na^+-P_i-COT increases, but the property of a single Na^+-P_i-COT remains the same. (B) Stimulus increases the synthesis of mRNA coding enzymes in the processing of Na^+P_i-COT. The total number of Na^+P_i-COT will not change, but Na^+-P_i-COT with altered properties (V_{max}, K_m) will result (Fig. 2). A combination of the two mechanisms is also possible. As with rapid stimuli, one agent may act differently on the proximal convoluted tubule and pars recta. For example, whereas T_3 may increase biogenesis of the Na^+-P_i-COT in the pars recta, it may increase biogenesis of the Na^+-H antiporter in the proximal convoluted tubule [60].

Specific factors

Factors which induce a specific change in BBM P_i transport only after 24 hr or longer are hormones such as thyroxine, T_3 [60, 61], glucocorticoids [59, 62, 63], and vitamin D [64], and metabolic stimuli of a complex nature, such as metabolic acidosis [65, 66], chronic "adaptive" feeding of Low P_i diet [67, 68] and starvation [69, 70] (Table 2). The changes in transport kinetics induced by these factors appear to be dependent on the nature of the stimulus. Most of the stimuli induce a change only in the V_{max}, but notable exceptions are starvation, which changes only the K_m [70], and thyroxine, which changes both the V_{max} and the K_m [61].

Physiological replacement doses of 1,25-dihydroxyvitamin D_3 administered to vitamin D deprived rats stimulated BBM P_i transport within 24 hr [64]. There is evidence, however, that a time interval of 24 hr may not be necessary. For example, infusion of an equivalent dose produced the same effect after 6 hr [71], and a pharmacological dose administered to rachitic chicks stimulated P_i uptake by renal cells within 3 hr [72]. Studies *in vitro* demonstrated that the rapid effects of 1,25-dihydroxyvitamin D_3 were prevented by protein synthesis inhibitors [59], which is consistent with an effect on transporter biogenesis although the time course is apparently more rapid than that of other factors in this group.

The relatively slow response to this group of stimuli has facilitated investigations of how the different stimuli may interact. The effects of adaptation to a low phosphorus diet are reversed by subsequent

Table 2. Summary of factors producing long-term adaptive changes in BBM transport of P_i

Factors	Principal characteristics of adaptive induced response
Thyroid hormones Glucocorticoids Vitamin D Metabolic acidosis Chronic dietary P_i deprivation Fasting	Change in BBM P_i transport occurs after 1–2 days and may involve V_{max} and/or the K_m. It is prevented by inhibitors of *de novo* protein synthesis.

starvation [69, 70], chronic metabolic acidosis [65] or treatment with glucocorticoids [62]. Starvation reverses the adaptation by inducing an increase in the K_m for Na^+-dependent BBM P_i transport, whereas reversal by both metabolic acidosis and glucocorticoids involves a decrease in the V_{max}. The lack of effect of chronic respiratory acidosis on renal P_i transport [73] suggests that the effects of chronic metabolic acidosis are not due simply to a change in systemic pH. In addition to the changes in BBM P_i transport, this group of stimuli produces marked changes in the rate of renal gluconeogenesis. Whether the changes in gluconeogenesis are a secondary effect or are causally related to the adaptive changes in P_i transport has not been determined.

Recent studies describing the effects of thyroid hormones [60, 61] have demonstrated a marked and specific stimulatory effect on BBM transport of P_i which occurs independently of any changes in diffusional Na^+ uptake and BBM uptake of other solutes [60, 61]. The apparently additive stimulatory effects of T_3 and chronic P_i deprivation [60] have focused attention on the differential response of BBM from proximal tubules located in superficial cortex and deep cortical zone. The stimulatory effect of feeding low phosphate diet to dogs was found to be restricted to BBM vesicles from superficial nephrons [50]. Similar response to dietary P_i restriction was observed also in rat kidney (S. T. Turner and T. P. Dousa, unpublished observations), while T_3 stimulated P_i transport only in BBM vesicles derived from juxtamedullary nephrons [60]. It appears that, in rat kidney, BBM vesicles prepared from juxtamedullary cortex and outer stripe of red medulla [60] are likely enriched in membranes mainly from late proximal tubules—pars recta (A. N. K. Yusufi and T. P. Dousa, unpublished observations). Thus, it is a different site of action rather than a different mechanism which is the likely explanation for the additive effects of these stimuli. As discussed above, certain rapidly acting factors (PTH and calcitonin) also act preferentially on a specific nephron subpopulation.

The concept that thyroid hormones regulate BBM P_i transport through an effect on biogenesis of the transporters is consistent with the observations that inhibition of protein synthesis or disruption of microtubules [18, 56] prevented the changes in P_i transport. An important related finding is the presence in renal tissue of both cytosolic and nuclear binding sites for thyroid hormones [74, 75]. Proximal tubule is a specific site of conversion of T_4 to its more active form, T_3 [76].

SUMMARY

The wealth of new information on BBM transport of P_i which has accumulated in recent years gives an indication of the importance and intellectual challenge that the mechanism of this process poses to investigators. In this brief reflection on the field, we have tried to draw attention to some general principles and features which may be helpful as working hypotheses in the development of the field. To date, a disproportionate amount of effort may have been spent on deciphering putative intracellular regulatory mechanisms, without knowing some essential fundamental properties of the Na^+-P_i-COT. We suggest that a major effort should be exerted towards elucidating biogenesis of the Na^+-P_i-COT, the possible existence of a membrane cycling mechanism, and a refined analysis of the Na^+-P_i-COT in specific subsegments of proximal tubules. Advances in these areas together with studies of both the rapid and long-term adaptive regulation of P_i transport are needed, given the central role of the kidney in total body P_i homeostasis both in health and disease.

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