of the vascular structure. Binding to adhesion molecules regulates the migration of leukocytes from the blood stream into the vessel wall inflammatory diseases [4]. Adhesion molecules seem also to play a role in the migration of endothelial cells into the vessel wall during angiogenesis, as well as the migration of vascular smooth muscle cells from the media into the intima in chronic vascular disease. The surface expression of the adhesion molecules is regulated by intracellular free calcium concentration and protein kinase C. We studied the effects of hyperlipoproteinema and hyperglycemia, known risk factors of chronic vascular disease, on the surface expression of adhesion molecules on endothelial cells [12]. We could show that different isozymes of the protein kinase C family are responsible for phosphorylating and regulating the adhesion molecules [13]. We also addressed the question whether or not binding to adhesion molecules affects the intracellular signalling cascade and could show that binding to the selectin family of adhesion molecules triggers tyrosine phosphorylation in white blood cells [14].

Using FACS-analysis, we assessed the expression of adhesion molecules on leukocytes in patients with Wegener's Granulomatosis. We found that during exacerbation of the disease there is increased expression of the integrin receptors LFA-1 and VLA-4 on granulocytes and monocytes in these patients [11]. Influencing expression of adhesion molecules could be an important therapeutic approach in inflammatory vascular diseases, as well as in chronic vascular diseases. We are therefore investigating the possibilities of an antisense strategy for inhibition of adhesion molecule expression. For the gene transfer, virus-coated liposomes (Sendai virus) are used in cell cultures and in animal models. The aim of these studies is to interfere with the complex alterations in inter- and intracellular signalling in chronic diseases of the vascular wall and to try to re-establish undisturbed cellular communication. In short, our laboratory is dedicated to establish 'who is talking to whom' in the vascular wall. We have encountered inappropriate silence and unacceptable shouting matches between the components. Our goal is the promotion of mutual understanding through reasonable communications.

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Molecular mechanisms in renal phosphate reabsorption

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Plasma levels of inorganic phosphate (P_i) are mainly controlled by altering the rate of its proximal tubular reabsorption [1]. The cellular mechanisms involved in the proximal tubular handling of P_i have been characterized by *in vivo* and *in vitro* microperfusion techniques, by tissue culture experiments, and by studies with isolated brush border and basolateral membrane vesicles [1,13,14]. Uptake of P_i from the tubular lumen into the epithelial cell is via sodium coupled P_i-trans-

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port (Na/P_i-cotransport) mediating 'uphill' transport of P_i. Transport across the basolateral membrane is 'equilibrating' involving multiple pathways, e.g. an anion exchange mechanism and a Na/P_i-cotransporter (different from that/those in the apical membrane) [13,14]. The transport mechanisms located in the apical membrane are functionally and structurally well defined (see below); the knowledge on the basolaterally located transporters is rather limited. Physiological regulation of proximal tubular P_i-reabsorption is most likely entirely related to alterations in the function of the apical transporter(s) [13,14].

Molecular characteristics of brush border membrane Na/P_i-cotransport

In recent years we have isolated by an expression cloning strategy different cDNAs encoding apical Na/P_i-cotransporters [11,15,19]. The key observation in the cloning experiments was the increased uptake of P_i in *Xenopus laevis* oocytes after injection of kidney cortex mRNA [e.g. 11,19]. Cloning procedures led to the functional identification of two different cDNA's: The originally isolated NaP_i-1 cDNA was obtained from a rabbit kidney cortex cDNA library [19]; later a cDNA from a rat (NaP_i-2) and a human kidney cortex library (NaP_i-3) was isolated [11]. NaP_i-2- and NaP_i-3-proteins are highly homologous but different from the NaP_i-1-protein [15]. Accordingly, two structurally different Na/P_i-cotransporters have been identified: Type I (NaP_i-1) and Type II (NaP_i-2/3). Subsequently homologs to either NaP_i-1 or NaP_i-2/3 have been isolated from a variety of species; interestingly, the two transporters seem to coexist in the kidney cortex of several species [15,18].

Functional characteristics

As the Type I (NaP_i-1) and Type II (NaP_i-2/3) transporters have been obtained by functional screening (increasing Na-dependent P_i-uptake) both proteins are by definition Na/P_i-cotransporters. Nevertheless, extensive further characterization of their transport functions were performed. For the Type II transporters a high selectivity for P_i, a 3:1 stoichiometry ($Na:P_i$) and a strong pH-dependence (higher rates at more alkaline pH-values) were found in tracer and electrophysiological studies [2,15]. For the Type I transporter no particular pH dependence was observed. Furthermore, recent electrophysiological studies have suggested that the Type I transporter might also transport different organic anions (A. Busch *et al.*, unpublished). A comparison of the functional properties of Type I and Type II transporters expressed in oocytes with transport characteristics known from studies on isolated brush border membrane vesicles suggests that the Type II transporter is mainly responsible for the 'apparent' properties of brush border Na/P_i-cotransport.

Tissue localization

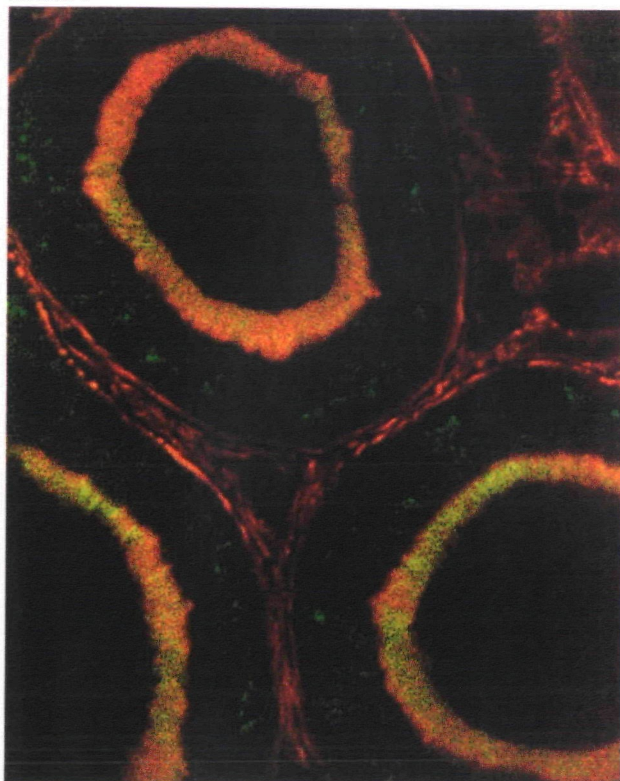
The cloning producure started from mRNA isolated from kidney cortex. Thus, it was necessary to document that the corresponding proteins (NaP_i-1; NaP_i-2) are present in proximal tubular epithelial cells and there in the apical membrane. For the Type I and Type II transporters we could show that the mRNA encoding these transporters are present in proximal tubular cells and that the related proteins are located in the brush border membrane [4,5,15; see also Figure 1]. Together with above functional properties we conclude that the Type II transporter is indeed the brush border membrane Na/P_i-cotransporter; the Type I transporter is a brush border transporter with broader specificity.

Structural properties

The Type I transporters are about 465 and the Type II transporters are 635 amino acids in length [11,15,19]. Both transporters have several transmembrane spanning regions (Type I: ~9; Type II: ~8) and are glycosylated [6,15]. Both transporters contain consensus sequence motifs for protein phosphorylations [mostly kinase C sites; 7,11,15,19]. In studies on rat brush border membrane vesicles we could show by immunoprecipitation experiments that the NaP_i-2 protein is a phosphoprotein [7]; such phosphorylation reactions could be involved in protein kinase mediated regulation of the transporter (see below).

Physiological regulation

Proximal tubular P_i-reabsorption, and thus brush border membrane Na/P_i-cotransport, is regulated by a variety of hormonal and non-hormonal factors [1,13,14]. We will only discuss briefly two examples: Parathyroid hormone (PTH), as a known phosphaturic hormone, mediates its inhibitory action on Na/P_i-cotransport by activating a complex intracellular regulatory machinery; the initial step is interaction with a hormone receptor capable of activating the adenylate cyclase (protein kinase A) and the phospholipase-C (protein kinase C) pathways [12]. The Type II Na/P_i-cotransporter is a target for PTH regulation [8]. If the rat II transporter is expressed in oocytes, Na/P_i-cotransporter activity is reduced by pharmacological activation of protein kinase C but not of protein kinase A [7]. However, we do not yet know whether this is particular for the oocyte system and which of the kinase sites might be involved in this regulation. In tissue culture experiments we have generated evidence for an involvement of an endocytic step in PTH-action [14]. In immunohistochemical studies we documented that infusion of PTH leads in rat proximal tubules to an immediate removal of apical Na/P_i-cotransporters and to their appearance in intracellular structures (also in subapical vesicles; Figure 1) [8]. It is the task of future experiments to determine the signalling events linking the PTH-receptor occupancy to withdrawal and inactivation of brush border membrane Na/P_i-



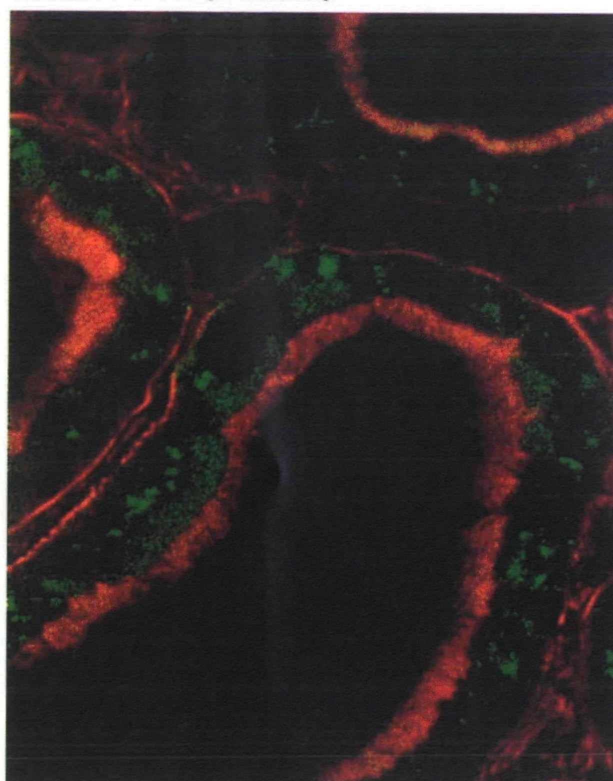


Fig. 1. Cross section through rat kidney cortex of control and PTH-infused animals. NaP_i-2 antibody stains selectively the Type II Na/P_i-cotransporter [4] and appears as green fluorescence. An antibody recognizing β-actin appears as red fluorescence. As seen in control animals the Type II transporter is located mostly at brush border sites and appears as 'yellow' ('colocalization' of β-actin and transporter). After PTH-treatment the transporter is removed from the brush border structure (red/β-actin) and appears now in intracellular compartments (green; mostly subapical endosomes) [8].

cotransporters. The activity of the Type II Na/P_i-cotransporter is also under tight control of dietary intake. Using molecular tools (antibodies; cDNA probes) we could show that acute regulation (1–2 h) in rats fed either high or low P_i-diets results in a different surface expression (brush border membrane) of the transporter protein most likely also involving steps of membrane traffic. On the other hand, chronic regulation ('adaptation', 4 h and more) involves also transcriptional and translational mechanisms [10]. It is evident that using the newly available molecular tools we will start to understand a variety of such regulatory mechanisms at a molecular level.

Genetic disorders

There are two well characterized inherited diseases with impaired tubular phosphate reabsorption [e.g. 16]. We could show in the mouse model for X-linked hypophosphatemic rickets (Hyp) that the Type II transporter (in terms of its function, specific transporter mRNA and protein) is reduced in affected animals [17]. However, the Type II transporter is not on chromosome X but on 5 [9]. Thus, the X-linked factor controls either the transcription rate (or mRNA stability) of the Type II transporter. The Type II transporter is a candidate for being directly involved in the autosomal form of hereditary hypophosphatemic rickets. The Type I transporter has been localized in the human to chromosome 6 [3]. On the basis of above discussed 'general' functional characteristics of the Type I transporter we do not think that it might play a crucial role in these genetic disorders. Future experiments (e.g. linkage studies and promoter/reporter gene studies) will certainly clarify in the near future the molecular mechanisms involved in the two described hereditary forms of hypophosphatemia.

Perspectives

The newly developed molecular probes for the major regulated Na/P_i-cotransporter of the brush border membrane offers now the possibility to study at the molecular level physiological and pathophysiological regulation of renal proximal tubular P_i-reabsorption. These studies will mainly go in three directions: (i) at the protein level to understand hormonal (acute, protein kinase mediated) regulation of the transporter including its membrane trafficking. (ii) At the level of the gene organisation in order to understand regulatory

mechanisms involving gene activation. (iii) At the level of genetic aberations in order to understand the hereditary deffects. As a part of the latter experiments gene knockout experiments (Type II transporter in mice) might be relevant to understand the mechanisms of pathophysiology of the different forms of hypophosphatemia.

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The fibroblast—a (trans-) differentiated cell?

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Introduction

Renal fibrosis is one of the hallmarks of chronic renal disease. It is characterized by excessive synthesis and deposition of extracellular matrix substances such as collagen types I and III, fibronectin, and proteoglycans. Fibroblasts are—as the name implies—the main effector cells in renal tubulointerstitial fibrosis. In 1867 Cohnheim published a classical paper on mechanisms of inflammation, stating that fibroblasts (contractile cellular elements as they were called at the time) are descendants of migrating leukocytes [1]. This theory was widely held until 1970 when Ross, using parabiotic rats, proved that fibroblasts are of local origin [2]. In fact, today it is widely assumed that all renal fibroblasts are derived from interstitial cells. However, recent research demonstrates that fibroblasts may originate from other cellular elements beside renal interstitial cells. Almost 130 years after Cohnheim new aspects of fibroblast origin are being discovered.

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Is the fibroblast a differentiated cell?

Apart from the collecting duct cells, which are descendants of the ureteric epithelium, all kidney cells are