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Chloride Conductance and P_i Transport are Separate Functions Induced by the Expression of NaPi-1 in *Xenopus* Oocytes

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Abstract. Expression of the protein NaPi-1 in *Xenopus* oocytes has previously been shown to induce an outwardly rectifying Cl^- conductance (G_{Cl}), organic anion transport and Na^+-dependent P_i-uptake. In the present study we investigated the relation between the NaPi-1 induced G_{Cl} and P_i-induced currents and transport. NaPi-1 expression induced P_i-transport, which was not different at 1–20 ng/oocyte NaPi-1 cRNA injection and was already maximal at 1–2 days after cRNA injection. In contrast, G_{Cl} was augmented at increased amounts of cRNA injection (1–20 ng/oocyte) and over a five day expression period. Subsequently all experiments were performed on oocytes injected with 20 ng/oocytes cRNA. P_i-induced currents (*I*p) could be observed in NaPi-1 expressing oocytes at high concentrations of P_i ($\geqslant$ 1 mM P_i). The amplitudes of *I*p correlated well with G_{Cl}. *I*p was blocked by the Cl^- channel blocker NPPB, partially Na^+-dependent and completely abolished in Cl^- free solution. In contrast, P_i-transport in NaPi-1 expressing oocytes was not NPPB sensitive, stronger depending on extracellular Na^+ and weakly affected by Cl^- substitution. Endogenous P_i-uptake in water-injected oocytes amounted in all experiments to 30–50% of the Na^+-dependent P_i-transport observed in NaPi-1 expressing oocytes. The properties of the endogenous P_i-uptake system (K_m for P_i > 1 mM; partial Na^+- and Cl^--dependence; lack of NPPB block) were similar to the NaPi-1 induced P_i-uptake, but no *I*p could be recorded at P_i-concentrations $\leqslant$3 mM. In summary, the present data suggest that *I*p does not reflect charge transfer related to P_i-uptake, but a P_i-mediated modulation of G_{Cl}.

Key words: Cl^- Channel — Organic Anions — Phosphate — NPPB

Introduction

Renal proximal tubule cells play a major role in the reabsorption and excretion of electrolytes, minerals and other physiological substrates as well as xenobiotics. Two proximal tubular brush border proteins with distinct molecular structures have been identified by expression cloning strategies (type I and type II Na^+/P_i-cotransporter) and shown to induce Na^+-dependent transport of inorganic phosphate (P_i) [14, 16, 17, 27]. The type II Na^+/P_i-cotransporter expressed in *Xenopus* oocytes meets all known characteristics of renal brush border membrane Na^+/P_i-cotransport, such as sigmoidal Na^+-dependence, pH-sensitivity and high P_i-affinity (apparent K_m of 0.1 mM); [5, 6, 9, 14]. It is altered at the functional and molecular level under a variety of physiological and pathophysiological conditions [11, 12, 22, 23, 25, 26; for review *see:* 16, 17]. Whereas the role of type II transporter in P_i absorption is well defined, the physiological function of the type I transporter, which lacks above landmarks of P_i transport, is still unclear. NaPi-1 cRNAs have been cloned from different species including human, mice and rabbits [7, 15, 27]; also a NaPi-1-homologue from human brain has been identified [18, 19, 20]. The amino acid sequences of NaPi-1 and NaPi-2 are not related, the similarity between both sequences is only about 10–15% (for review *see:* 16, 17). Hybrid depletion experiments on type II and type I related Na^+-dependent P_i-uptake activity after oocyte in-

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jection experiments indicated that type II transporter related P_i-uptake is more important than that of type I (about 3:1; [15]). This might explain the correlation of the overall transport properties of the type II transport activities observed after injection into oocytes with those observed in brush border membranes (*see above*). Furthermore, it has recently been shown that the type I Na^+/P_i-cotransporter in rats is differentially regulated than the type II transporter and might only play a housekeeping role in P_i-absorption [13]. We could recently show that expression of NaPi-1 in *Xenopus* oocytes induced a Cl^- conductance, also permeable for other halides and the organic anion benzylpenicillin [4]. This conductance was inhibited by classical Cl^- channel blockers, such as niflumic acid and NPPB [4, 10].

The focus of the present study was to analyze in detail a potential interrelationship between the two above recorded activities (Na^+-dependent P_i-uptake and anion conductance) observed after expression of NaPi-1 in oocytes. The data suggest that the two activities are not expressed in a coordinate manner suggesting that the apparent bifunctional properties might in part be related to the modulation of intrinsic oocyte transport properties rather than directly related to NaPi-1-mediated transport.

Materials and Methods

Electrophysiological Measurements

cRNA encoding NaPi-1 was synthesized in vitro as previously described [27]. Dissection of *Xenopus laevis* ovaries, collection and handling of the oocytes has been described in detail [3]. All experiments were performed on oocytes injected with 20 ng cRNA/oocyte on day 1–5 after cRNA-injection, if not stated otherwise. Two-electrode voltage-clamp recordings were performed at 22°C using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA) and MacLab D/A converter and software for data acquisition and analysis (ADInstruments, Castle Hill, Australia). The external control solution (ND 96) contained (in mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES (titrated with NaOH to pH 7.4). For the determination of *I*p sodium phosphate was added to the ND96 solution resulting in a final composition of (in mM): 96 NaCl, 3 Na-P_i (total: mono- and divalent), 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES; pH 7.4. In some experiments all external Cl^- was substituted by gluconate, phosphate or sulfate. In the latter two solutions $CaCl_2$ was omitted to avoid precipitation of calcium salts, the phosphate substituted solution had a final Na-P_i (total: mono- and divalent) concentration of 69 mM, the sulfate substituted solution a final Na_2SO_4 concentration of 63.9 mM. Because of its Ca^{2+}-chelating capacity, the free Ca^{2+} concentration in the gluconate containing solutions was <0.1 mM. Because it was previously shown that Ca^{2+} does not affect the NaPi-1-induced anion conductance [4], the decrease in Ca^{2+} was not compensated. For the examination of the Na^+ dependence, Na^+ was replaced by equimolar concentrations of choline in the external solution. The flow rate of the superfusion was 20 ml/min and a complete exchange of the bath solution was reached within about 10 sec.

Determination of G_{Cl}

The total conductance of oocytes was determined by performing voltage ramps (duration was 2 sec) from −80 to 40 mV (holding potential was −40 mV). The NaPi-1 induced anion conductance is almost completely blocked at 100 μM NPPB [4]. At the end of every experiment we perfused therefore the oocytes with 100 μM NPPB. G_{Cl} reflects the total conductance subtracted with the NPPB-resistant conductance. The NPPB-resistant conductance is mainly a leak through the oocyte membrane. Oocytes exerting a NPPB-resistant conductance > 0.5 μS were rejected, because they were considered *unhealthy*.

Uptake Studies

Uptake studies with $[^{33}P]HPO_4^-$ were performed in 5-ml polypropylen tubes containing 7 oocytes for each determination 1–5 days after NaPi-1 cRNA injection. Oocytes were washed twice with 4 ml OR2+ transport buffer (in mM: 82.5 NaCl, 2.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4, 5 HEPES pH 7.8) and incubated for 30 min in 100 μl OR2+ containing different amounts of HPO_4^{2-} and $[^{33}P]HPO_4^{2-}$ resulting in a specific activity of 10–200 dpm/pmol. Afterwards oocytes were washed three times with 4-ml ice-cold OR2+, removing external radioactivity. Oocytes were placed in scintillation vials and radioactivity was measured by liquid scintillation counting.

Chemicals used were NPPB (5-Nitro-2-(3-phenylpropylamino) benzoic acid; gift from Dr. R. Greger, University of Freiburg) and $[^{33}P]HPO_4^{2-}$ (Amersham), all other chemicals were of analytical grade and purchased from commercial suppliers. All data are given as means ± SEM where *n* indicates the number of experiments or oocytes. The current sizes given in this report varied, depending on the time after and amount of cRNA injection and on the batch of oocytes (from different animals). Therefore, throughout the paper we show experimental data obtained with one particular batch of oocytes. All sets of experiments have been repeated at least once with a different batch of oocytes; in all repetitions qualitatively similar data have been obtained.

Results and Discussion

Effect of Amount of cRNA and Time of Expression on G_{Cl} and P_i-Uptake

Previously we have shown that injection of NaPi-1 cRNA higher than 2 ng/oocyte and time of expression >36 hr leads to a decrease in the NaPi-1 induced Na^+-dependent P_i-uptake properties [27]. On the basis of this observation we had concluded that overexpression of the NaPi-1 protein might not be tolerated by the oocyte. Alternative explanations are that the NaPi-1 induced transport activity is downregulated at high protein density or that NaPi-1 is regulating some intrinsic activity and that this effect would be saturated already at this low level of expression. To address this question more directly we have injected different amounts of cRNA and allowed for different time of expression (Fig. 1). In agreement with our previous studies [27] we observed 1–2 days after cRNA injection the highest level of NaPi-1 induced P_i-uptake with rather low amounts (1 ng/oocyte) of cRNA. At all days NaPi-1 expression induced Na^+/P_i cotransport which was 2–4 fold higher than the endogenous transport activity [6, 14, 16, 17, 27], but not dependent on the amount of injected cRNA (Fig. 1*A*). For example, at 1 and 20 ng cRNA/oocyte P_i-transport

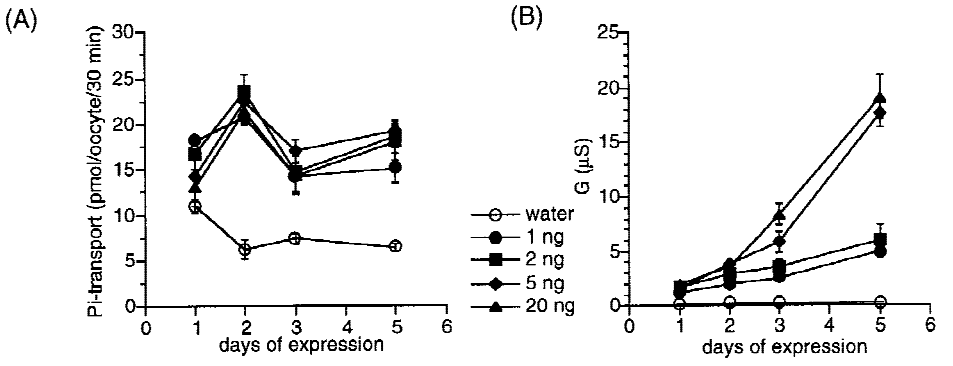

Fig. 1. Dependence of P_i-uptake and G_{Cl} in *Xenopus* oocytes on the amount of NaPi-1 cRNA injection and time of NaPi-1 expression. P_i-uptake (*A*) and G_{Cl} (*B*) were determined as described in Materials and Methods. The data represent means ± SEM ($n = 7$).

(pmol/oocyte/30 min) was 18.2 ± 0.5 and 13.0 ± 2.0 at day 1, respectively, and 15.1 ± 1.6 and 18.0 ± 2.0 at day 5, respectively ($n = 7$). In Fig. 1*B* the comparable set of data is shown for the NaPi-1 induced Cl-conductance (G_{Cl}). In contrast to P_i-uptake, G_{Cl} increases with increased amounts of injected cRNA and time of expression. After the injection of 1 ng cRNA/oocyte G_{Cl} was 1.16 ± 0.08 μS and 4.94 ± 0.27 μS at days 1 and 5, respectively, and at 20 ng cRNA/oocyte G_{Cl} was 2.03 ± 0.10 μS and 19.11 ± 1.99 μS, respectively, at these days.

Characteristics of NaP*i*-1 Induced G_{Cl} and *I*p

We described previously that NaPi-1 expressing oocytes exerted P_i-induced currents (*I*p) only at high P_i-concentrations [4]. *I*p did not seem to be saturated at 3 mM P_i, i.e., higher P_i concentrations resulted in elevated *I*p. Due to the small signal, however, the concentration dependence could not be evaluated. Here, we wanted to compare the properties of *I*p with the NaPi-1 induced conductance, G_{Cl}. Inwardly, directed holding currents ranging from approximately −0.1 to −1 μA at days 2 to 6 of NaPi-1 expression (after injection of 20 ng cRNA/oocyte), respectively, were observed upon voltage-clamping *Xenopus* oocytes at a holding potential of −50 mV ($n = 52$). At this potential superfusion with P_i concentrations ⩾1 mM were required to induce significant additional *I*p deviating from the holding current (Fig. 2*A*). The background conductance was an outwardly rectifying Cl^--conductance reversing its current direction at −31.1 ± 1.2 mV in voltage ramps (Fig. 2*A; n* = 19), similar to what was previously described [4]. In the same batch of oocytes we recorded Cl^- conductance and *I*p in individual oocytes over an expression period of 6 days. Similar as shown above, the amplitude of both G_{Cl} and *I*p depended on the time of NaPi-1 expression, i.e., they were smallest at day 1 and largest at day 6 of NaPi-1 expression (*data not shown*). Plotting *I*p from all individual experiments *vs.* G_{Cl}, a correlation was observed

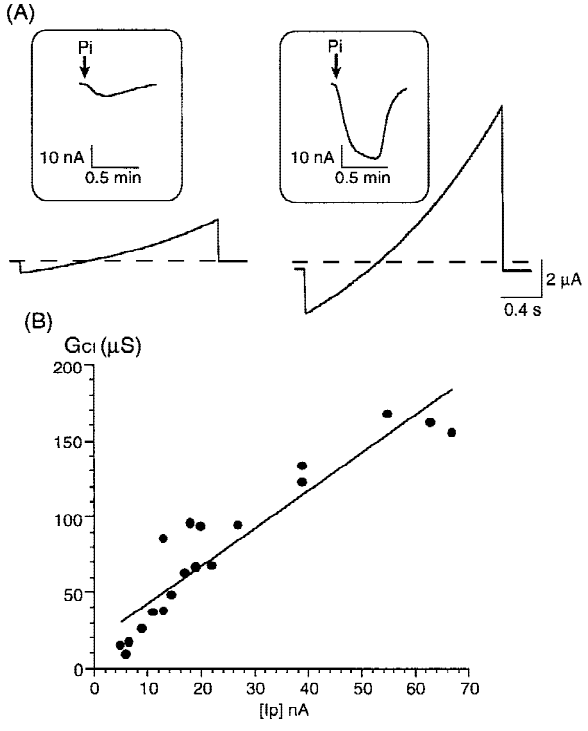

Fig. 2. Correlation of NaPi-1 induced Cl^- currents and P_i induced currents. (*A*) Cl^- currents during voltage ramps (duration was 2 sec) from −80 to 40 mV (holding potential was −40 mV) in the absence of P_i. *I*p (insert) was recorded at a holding potential of −50 mV during a 30-sec superfusion with P_i (3 mM). The traces for *I*p (inserts) and Cl^- currents were recorded at days 2 (left) and 6 (right) after cRNA injection (20 ng/oocyte). (*B*) The diagram shows the recorded Cl^- currents as a function of Ip.

(Fig. 2*B; n* = 19). In general it can be stated that the larger G_{Cl} the larger was *I*p. The size of G_{Cl} did not allow a proper determination of the reversal potential of *I*p, however, it was not in the range of the reversal potential of a Na^+-dependent conductance. In all recordings from water-injected oocytes ($n = 25$) *I*p (at 3 mM P_i) was smaller than −3 nA and not further analyzed.

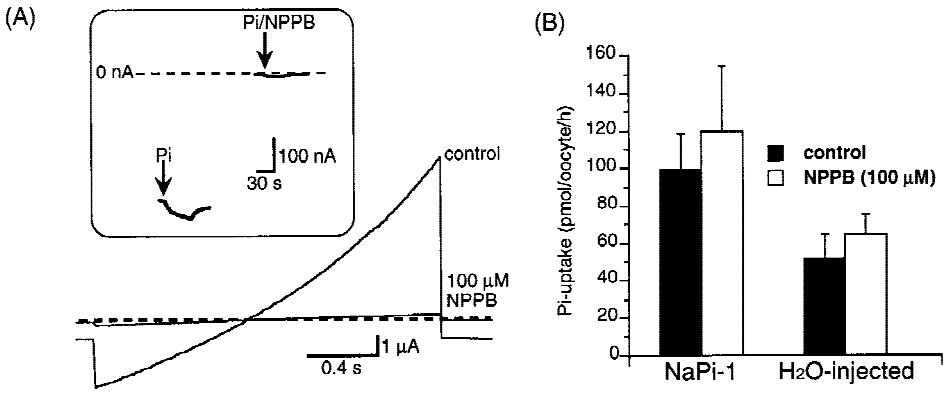

Fig. 3. Effects of NPPB on G_{Cl}, *I*p and P_i-uptake. (*A*) Inhibition of *I*p (insert) and Cl^- currents in NaPi-1 expressing oocytes by the Cl^- channel blocker NPPB (100 µM). Both currents were recorded as described for Fig. 2. Insert: *I*p in the absence or presence of NPPB (100 µM), which abolishes both the large inward holding current and the P_i induced current. The dashed line indicates 0 current. (*B*) Effects of NPPB (100 µM) on P_i-uptake in NaPi-1 expressing and water-injected oocytes.

Effects of NPPB, Na^+ and Cl^- on G_{Cl}, *I*p and P_i-Uptake

The NaPi-1 induced Cl^--conductance has been shown to be inhibited by the Cl^- channel blocker NPPB with an IC_{50} of 3 µM [4]. We therefore performed conductance- and *I*p-recordings in the presence of 100 µM NPPB (recordings were performed on day 4 after injection of 20 ng cRNA/oocyte; Fig. 3*A*). Both the Cl^- conductance and *I*p were greatly reduced to 9.7 ± 1.1% ($n = 7$) and 14.9 ± 2.7% ($n = 7$) of control, respectively ($P = 0.126$). Due to the small signal, however, the concentration-response curve could not be evaluated. In contrast, uptake of radiolabeled P_i in NaPi-1 expressing oocytes (day 3 after injection of 20 ng cRNA/oocyte) was not significantly affected by 100 µM NPPB (Fig. 3*B; n* = 7 per group of oocytes) demonstrating a striking difference of *I*p and P_i-uptake pharmacology.

Substitution of extracellular Na^+ with choline had no significant effect on the Cl^- conductance and on *I*p (at 3 mM P_i). G_{Cl} was 27.2 ± 2.0 µS and 30.0 ± 1.9 µS at control and 0 Na^+ ($n = 6$), respectively ($P = 0.334$). *I*p was −14.4 ± 1.8 nA and −11.5 ± 3.2 nA at control and 0 Na^+ (Fig. 4*A; n* = 6), respectively ($P = 0.4$). In uptake in NaPi-1 expressing oocytes [^{33}P]HPO_4^- (1 mM) transport was reduced by 75% in the absence of Na^+ (Fig. 4*B; n* = 7 per group of oocytes). The lack of sensitivity of *I*p contrasts the clear Na^+-dependence of P_i-uptake and further supports the hypothesis that *I*p does not reflect Na^+-dependent P_i-uptake.

The Na^+-dependence of NaPi-1 mediated P_i-transport has been already demonstrated before [27], but a possible Cl^--dependence has not been analyzed. Because NaPi-1 induces a Cl^- conductance, we tested whether *I*p and [^{33}P]HPO_4^--uptake were affected by alterations in extracellular Cl^-. These recordings were performed at day 3 after injection of 20 ng cRNA/oocyte. As described previously [4], deletion of extracellular Cl^- reduced the conductance by over 90% (*data not shown; n* = 12). Figure 5 shows the effects of Cl^- substitution by gluconate on *I*p and P_i-uptake. Substitution of extracellular Cl^- abolished *I*p completely (Fig. 5*A; I*p decreased from −11.9 ± 2.6 nA to −0.1 ± 1.2 nA; $n = 7$). In contrast, removal of extracellular Cl^- decreased uptake of [^{33}P]HPO_4^- (0.5 mM) only by half (from 61 ± 6 pmol/30 min under control to 29 ± 5 pmol/30 min under Cl^--free conditions; Fig. 5*B; n* = 7). This difference in Cl^- dependence of *I*p and P_i-uptake further supports that different mechanisms are underlying *I*p and P_i-uptake.

NaP*i*-1 Induced P_i-Transport Shares General Properties with an Endogenous P_i-Transport System in *Xenopus* Oocytes

As can be seen in Figs. 1 and 3–6 there was a substantial endogenous P_i-uptake system present in *Xenopus* oocytes which produced up to 50% of the P_i-uptake in NaPi-1 injected oocytes. Furthermore, the endogenous P_i-uptake displayed similar characteristics as the NaPi-1 induced P_i transport. The endogenous P_i-uptake was only partially Na^+-dependent (Fig. 4*B; n* = 7), partially Cl^--dependent (Fig. 5*B; n* = 7) and not inhibited by 100 µM NPPB (Fig. 3*B; n* = 7).

Finally, we determined the apparent affinity for P_i in both water-injected and NaPi-1 expressing oocytes (at day 3 after injection of 50 nl water or 10 ng Nai-1 cRNA/oocyte; Fig. 6; every point represents the mean and SEM of 7 oocytes). The data could be fitted by a Michaelis-Menten kinetic which gave apparent K_m values for P_i of 1.1 ± 0.1 mM (V_{max} was 91.1 ± 2.8 pmol/oocyte/h) and 1.4 ± 0.5 mM (V_{max} was 56.8 ± 7.0 pmol/oocyte/h) for

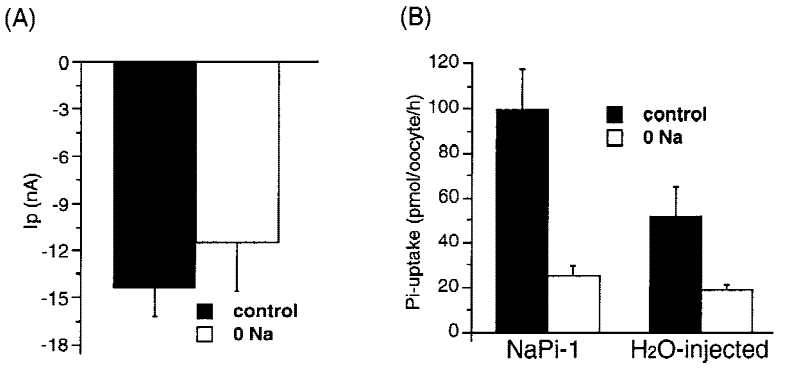

Fig. 4. Effects of Na^+ on *I*p (*A*) and uptake of P_i (*B*). (*A*) *I*p was not significantly affected by Na^+ substitution. (*B*) P_i-uptake at 1 mM P_i is significantly smaller in the absence of Na^+ in both NaPi-1 expressing and water-injected oocytes.

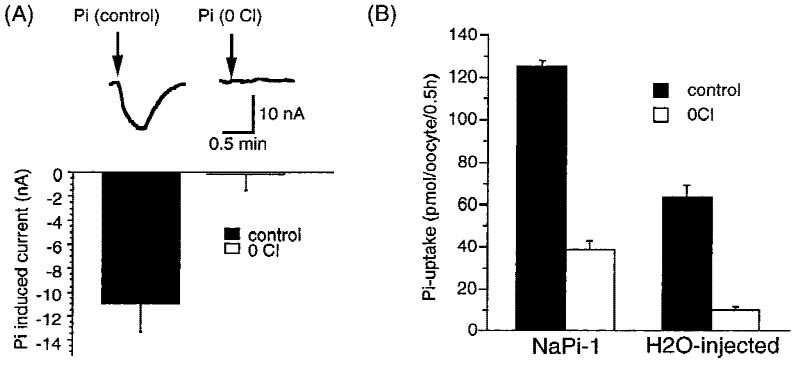

Fig. 5. Currents induced by P_i and uptake of P_i in NaPi-1 expressing oocytes in the presence and absence of extracellular Cl^-. (*A*) *I*p was induced by a 30-sec superfusion (start is indicated by the arrows) with 3 mM P_i at −50 mV. (*B*) P_i-uptake is reduced in the absence of Cl^- in both NaPi-1 expressing and water-injected oocytes.

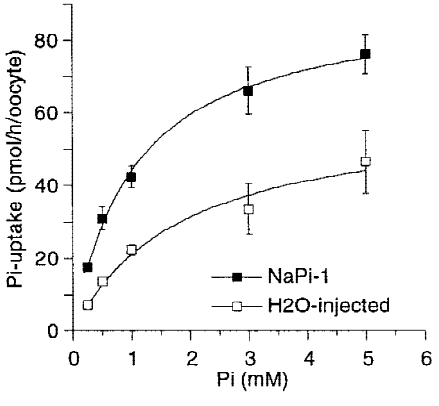

Fig. 6. Kinetics of P_i-uptake in NaPi-1 expressing and water-injected oocytes. The data were fitted to a Michaelis-Menten kinetic.

NaPi-1 expressing and water-injected oocytes, respectively. These K_m values for P_i-transport in NaPi-1 expressing or water-injected oocytes were not significantly different, but significantly differed from the concentration dependence of *I*p. In summary, the NaPi-1 induced P_i-transport corresponds in all properties to the endogenous P_i-transport system and both also correlate in their transport affinity (K_m). These findings could therefore indicate that NaPi-1 expression may upregulate an P_i-transport system which is endogenous to the *Xenopus* oocyte.

'Relation' Between *I*p, G_{Cl} and Na^+-Dependent P_i-Uptake

It is obvious that *I*p displays the same sensitivity to manipulations of the extracellular solution as G_{Cl}. Maneuvers that abolish G_{Cl} (superfusion with NPPB or deletion of Cl^-) also suppressed *I*p. Additionally, both are basically insensitive to changes in extracellular Na^+. Technical limitations, i.e., the very little *I*p (usually around −10 nA) in the presence of the almost 100-fold greater Cl^- conductance did not allow more detailed studies of *I*p. The P_i-conductance of NaPi-1 could be determined after replacement of chloride by phosphate. The G_{Pi} came to 35% of G_{Cl}; similarly, replacement of chloride by sulfate resulted in a G_{SO4} which amounted to 28% of G_{Cl}. Replacement of chloride by both anions resulted also in a shift of the reversal potential, supporting the idea that NaPi-1 induces an anion conductance of low selectivity. The small P_i-dependent inward currents could therefore be explained by an inhibition of G_{Cl}. This mechanism would also explain the Na^+-independence of G_{Cl} and *I*p. However, it is clear that the P_i-induced current, *I*p, does not reflect Na^+-dependent P_i-uptake as they differ in their pharmacology, Na^+- and Cl^--dependence. Moreover, whereas *I*p correlates in its amplitude with G_{Cl}, Na^+-dependent P_i-uptake does not.

It is important to point out the consequences of simple technical modifications on the results of the pres-

ent paper. G_{Cl} is depending on the amount of cRNA-injection and time of NaPi-1 expression. Working within the common frame for expression of exogenous proteins in *Xenopus* oocytes (injection of 1–20 ng cRNA/oocyte for an expression period of 1–5 days) could result in 20-fold differences in G_{Cl}, where Na^+-dependent P_i-uptake was not significantly altered under these conditions. At the smallest injected amount of cRNA/oocyte (1 ng) Na^+-dependent P_i-uptake was already maximal, in perfect agreement with the original data published by Werner et al. [27]. These authors even reported a reduction of Na^+-dependent P_i-uptake at cRNA amounts >2 ng/oocyte and expression periods longer than 1 day. In the present study most experiments were carried out on oocytes injected with 10–20 ng cRNA/oocyte at days 3–4 after injection, which could account for the difference in P_i-uptake stimulation in this paper (2–3-fold stimulation of Na^+-dependent P_i-uptake in NaPi-1 *vs.* water-injected oocytes) compared to the original paper (27; 4–6-fold stimulation). However, both papers on stimulation of Na^+-dependent P_i-uptake by NaPi-1 (cloned from rabbit) result in very different findings compared to the paper by Miyamato et al., with the human homologue NPT-1 (which is 69% identical to NaPi-1). NPT-1 expression stimulated P_i-uptake over 50-fold with a more than 5-fold higher P_i-affinity as described in the present study [15]. It has not been analyzed yet, whether NPT-1 also induces a Cl^--conductance similar to NaPi-1.

What is the Physiological Function of NaP*i*-1?

All studies dealing with NaPi-1 or its homologues demonstrated the induction of Na^+-dependent P_i-uptake. This was not only observed after expression of NaPi-1 in *Xenopus* oocytes, but also in other expression systems, such as MDCK cells [21]. However, it has never been clarified, whether NaPi-1 is a functional Na^+/P_i-cotransporter itself, or whether it is an activator/regulator of a Na^+/P_i-transport system endogenous to the employed expression systems. The present data cannot exclude that NaPi-1 is a Na^+/P_i-cotransporter itself, but there are several results favoring the hypothesis that it modulates the activity of an intrinsic Na^+/P_i-transport system: (i) In MDCK-cells and in oocytes used there is an intrinsic Na^+/P_i-cotransport system; in the present study we can show that this system has very similar properties to the NaPi-1 additionally induced Na^+/P_i-uptake activity. (ii) The maximal Na^+/P_i-cotransport is already saturated at very low cRNA-injections (<1 ng/oocyte), a characteristic which has also been shown for the expression of other regulators of intrinsic systems in *Xenopus* oocytes [1, 24]. In contrast to its role for Na^+/P_i-cotransport, the role of NaPi-1 for the induction of a chloride/anion conductance appears somewhat clearer. NaPi-1 induces an outwardly rectifying Ca^{2+}-independent chloride conductance in *Xenopus* oocytes which is also permeable for other organic anions [4]. This anion conductance is (a) not observed endogenously in *Xenopus* oocytes and (b) its expression is proportional to the level of NaPi-1 expression, which is one important characteristic for functional channel/transporter subunits. The physiological role of this anion conductance in the brush border of renal proximal tubules remains however unclear. Preliminary studies showed that NaPi-1 expression in oocytes induces uptake of uric acid which could be inhibited by the uricosuric drugs probenecid and benzbromarone (*unpublished results*). The specificity and significance of this observation and the clarification of the physiological role of NaPi-1 will be the topic of further studies.

As indicated above the human homologue NPT-1 of the rabbit NaPi-1 shows after expression of cRNA in oocytes high expression of Na/P_i-cotransport activity with properties (apparent K_m-values for P_i) different from intrinsic uptake [15]. This opens the possibility that NaPi-1/NPT-1 homologues have the possibility of being multifunctional, representing either a transporter or channel itself or a regulator of such intrinsic activity. Differences in sequence/structure might determine which function is 'dominant', as exemplified by the comparison between the human NPT-1 and rabbit NaPi-1 homologues in the present study [15, 27]. Finally, it has to be indicated that this protein has a rather restricted expression: NaPi-1 was found to be preferentially expressed in liver and kidney and there in the brush border membrane of proximal tubules [2, 8, 27].

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