Ammonium transport by the colonic H\(^+\)-K\(^+\)-ATPase expressed in Xenopus oocytes

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The complementary DNA cloned from rat distal colon by Crowson and Shull (11) belongs to the cDNAs for the phosphorylating class of ion-motive ATPases (P-ATPase family) and shares a high level of molecular homology with cDNAs for two other members of the P-type, K\(^+\)-dependent ATPase family, the Na\(^+\)-K\(^+\)-ATPase and the gastric H\(^+\)-K\(^+\)-ATPase. The product of the colonic P-ATPase \(\alpha\)-subunit gene, when expressed in the Xenopus oocyte heterologous expression system, exhibits functional and pharmacological properties similar to those of the gastric H\(^+\)-K\(^+\)-ATPase or of the Na\(^+\)-K\(^+\)-ATPase. Like the gastric H\(^+\)-K\(^+\)-ATPase, it mediates H\(^+\)-K\(^+\) exchange, but unlike the gastric isoform, it is insensitive to Sch-28080 and is inhibited by millimolar concentrations of ouabain (10). Thus the pharmacological profile of the colonic H\(^+\)-K\(^+\)-ATPase is similar to that of the “ouabain-resistant” Na\(^+\)-K\(^+\)-ATPase, which is inhibited by high concentrations of the cardiac glycoside (28). By using the oocyte expression system, we have also recently shown that, like the Na\(^+\)-K\(^+\)-ATPase, the colonic H\(^+\)-K\(^+\)-ATPase can extrude Na\(^+\) from the cytosol, acting as both a Na\(^+\)-K\(^+\)-ATPase and a H\(^+\)-K\(^+\)-ATPase (9). More recently, it has been demonstrated that another of the nongastric H\(^+\)-K\(^+\)-ATPases, the human ATP1AL1-encoded protein, also acts as a Na\(^+\)-K\(^+\)- and H\(^+\)-K\(^+\)-transporting ATPase mediating primarily Na\(^+\)/K\(^+\) exchange rather than H\(^+\)/K\(^+\) exchange when expressed in HEK-293 cells (17).

As with the ATP1AL1 (17), the physiological role of ionic transport mediated by the colonic H\(^+\)-K\(^+\)-ATPase is difficult to delineate. Colonic H\(^+\)-K\(^+\)-ATPase is found mainly in the distal colon (11) and the distal part of the nephron (1). Its overexpression in kidney cells during a low-K\(^+\) diet is consistent with its involvement in renal K\(^+\) reabsorption (12, 18); in the distal colon, where it is constitutively expressed, it is not upregulated by a low-K\(^+\) diet (18), but dietary K\(^+\) depletion increases colonic K\(^+\) absorption (14, 25). The physiological relevance of Na\(^+\) transport by the pump has not been established. The presence of the colonic H\(^+\)-K\(^+\)-ATPase in the distal nephron and its mediation of H\(^+\) extrusion from the cell suggest that it could be involved in regulating the acid-base balance. However, this putative role is not firmly supported by the findings of previous reports, in particular by the lack of effect of metabolic acidosis on the colonic H\(^+\)-K\(^+\)-ATPase mRNA levels (12). Aldosterone status, which is often linked to K\(^+\) balance, does not change the expression of the colonic H\(^+\)-K\(^+\)-ATPase in the kidney (18). Functional studies of the role of the colonic H\(^+\)-K\(^+\)-ATPase in acid-base homeostasis of renal tubular cells are difficult to interpret, because of the segmental and cellular heterogeneities of the distal nephron, because of the lack of a specific inhibitor of the colonic H\(^+\)-K\(^+\)-ATPase, and especially because gastric H\(^+\)-K\(^+\)-ATPase, vacuolar H\(^+\)-ATPase, and Na\(^+\)-K\(^+\)-ATPase are also present in this part of the nephron. The powerful tool of genetically modified animals has not yet clarified the roles of the colonic H\(^+\)-K\(^+\)-ATPase in renal ionic transport and physiology: when fed either a normal or a low-K\(^+\) diet, transgenic mice with an inactivated colonic H\(^+\)-K\(^+\) \(\alpha\)-subunit gene have the same Na\(^+\) and K\(^+\) urinary excretion as control mice (25). Thus functional expression of the colonic H\(^+\)-K\(^+\)-ATPase promises more help in understanding its mechanistic, functional, and pharmacological properties and may also stimulate investigations with other models.

Because the colonic H\(^+\)-K\(^+\)-ATPase is mainly expressed in the renal medullary collecting duct (1, 23, 29) and in the colon, which are both involved in the net...
transport of ammonium, we looked for the involvement of this pump in NH₄⁺ transmembrane transport after its functional expression in Xenopus oocytes. Measurements of ⁸⁶Rb influx, intracellular pH (pHᵢ), and extracellular pH (pHₑ), and intracellular Na⁺ activity ([Na⁺]ᵢ) were consistent with the colonic H⁺-K⁺-ATPase-mediated transport of NH₄⁺.

METHODS

cRNA synthesis and expression in Xenopus laevis oocytes. cRNAs of the rat Na⁺-K⁺-ATPase β₂-subunit (34) and of the catalytic α-subunit of the colonic H⁺-K⁺-ATPase, α₁ (11), were synthesized with the SP6 RNA polymerase (Promega).

Oocytes were injected with 2 ng of the Na⁺-K⁺-ATPase β₂-subunit cRNAs to be used as control oocytes or were co-injected with 2 ng of β₂ cRNAs plus 10 ng of α₁ cRNAs to express the colonic H⁺-K⁺-ATPase (9, 10). Experiments were performed 2 days after cRNA injection (9, 10). The oocytes were incubated at 18°C in an amphibian Ringer solution containing (in mM) 85 NaCl, 1 KCl, 1 CaCl₂, and 1 MgCl₂, buffered at pH 7.4 with TES-NaOH and supplemented with 100 µM penicillin and 100 µg/ml streptomycin. As justified below, 10 µM ouabain, 5 mM barium, 10⁻⁴ M bumetanide, and 1 mM diphenylamine-2-carboxylic acid (DPC) were added to the Ringer during the 2-day incubation period ([Na⁺]ᵢ measurements) or during the experiments (⁸⁶Rb uptake measurements and pHᵢ and pHₑ measurements).

⁸⁶Rb uptake. The effect of extracellular NH₄Cl on K⁺ uptake by oocytes was tested by using ⁸⁶Rb as a K⁺ surrogate. Batches of 8–10 oocytes were incubated before the experiment for 15 min at room temperature in a K⁺-free assay solution containing (in mM) 90 NaCl, 1 MgCl₂, and 0.41 Ca(NO₃)₂, buffered at pH 7.4 with TES-NaOH. The assay solution contained 10 µM ouabain to inhibit the endogenous Na⁺-K⁺-ATPase, which is very sensitive to this inhibitor (6), and 5 mM barium to inhibit the oocyte membrane K⁺ conductance (8), because these transport systems mediate K⁺ influx and may also trigger NH₄⁺ influx into the oocyte (8). Because Na⁺-K⁺-2Cl⁻ symport and nonsynaptic cationic conductance also allow K⁺ and NH₄⁺ entry into the oocyte (4, 8, 20), they were inhibited by adding 10⁻⁴ M bumetanide and 1 mM DPC to the assay solution. Preliminary experiments showed that ch⁻-K⁺-ATPase mediates 80 or 95% of the total ⁸⁶Rb uptake in the absence or presence of these inhibitors, respectively.

Preliminary experiments also showed that ⁸⁶Rb uptake is linear for at least 12 min. The oocytes were therefore transferred to assay solutions (composition given above) containing 5 µCi/ml ⁸⁶Rb and various concentrations of NH₄Cl (at the expense of NaCl) for 12 min before counting. The assay solutions were supplemented with 200 µM KCl to only partially activate the colonic H⁺-K⁺-ATPase (Kₘ for K⁺ is reported to be 730 µM (10)), except in the experiments to determine the apparent pump affinity for NH₄⁺ (in which various concentrations of external KCl were added). The colonic H⁺-K⁺-ATPase was inhibited by adding 2 mM ouabain to the assay solution when necessary [whereas the endogenous Na⁺-K⁺-ATPase is sensitive to micromolar concentrations of ouabain (6), the colonic H⁺-K⁺-ATPase is only moderately sensitive to the cardiac glycoside (10)]. Individual oocytes were lysed with 100 µl of 5% SDS, mixed with 2 ml of scintillation medium, and counted.

pHᵢ and [Na⁺]ᵢ measurements. Simultaneous measurements of membrane potential (Vₘ), and of intracellular proton activity expressed as pHᵢ, or of [Na⁺]ᵢ, were performed with double-barreled (ion selective and conventional) microelectrodes. The fabrication of ion-selective microelectrodes and the compositions of their filling solutions have been described elsewhere (2). The H⁺ ionophore 95291 (Fluka) and the Na⁺ ionophore 71176 (Fluka) were used for pHᵢ and [Na⁺]ᵢ measurements, respectively. Before use, the microelectrodes were beveled in a microgrinder (De Marco Engineering). Their slopes (S) were determined before intracellular measurement by measuring the change in potential caused by a 10-fold change in Na⁺ or H⁺ concentration in the extracellular fluid; S was checked again after each puncture. The intracellular activity of ion i (Ai) was calculated from the relation Ai = Aref - 10⁻³ Vᵢ/Vref, where Aref is the activity (in mM) of ion i in the reference solution and Vᵢ/Vref is the measured electrochemical potential difference (in mV) for ion i.

For pH-selective microelectrodes, S was 54–57 mV (pH of the testing solutions: 7.4–8.4); no interference with NH₄⁺ was detected. For Na⁺-selective microelectrodes, S was 50–56 mV when changing extracellular Na⁺ concentration ([Na⁺]ₑ) from 100 to 10 mM. In this solution, 90 mM KCl was added to maintain the osmolarity and to take into account the slight interference of the high intracellular K⁺ activity with [Na⁺]ᵢ measurements (2).

Single oocytes were placed in a perfusion microchamber and were punctured by selective microelectrodes under stereomicroscopic control. The electrical circuit has been described elsewhere (2). Superfusate solutions (containing 10 mM ouabain, 5 mM BaCl₂, 10⁻³ M bumetanide, and 1 mM DPC) were delivered by a gravimetric system equipped with an electronic rapid-switch device.

pHᵢ measurements. pHₑ was measured as previously described (10, 19). Briefly, individual oocytes (β₂- or α₁-β₂-expressing oocytes) were placed in an oil-surrounded droplet (1 µl) of a weakly buffered (0.5 mM MOPS; pH 7.6) solution containing (in mM) 110 NaCl, 0.5 MgCl₂, and 0.5 CaCl₂, sometimes supplemented with 10 mM NH₄Cl; 10 µM ouabain, 5 mM BaCl₂, 10⁻³ M bumetanide, and 1 mM DPC were always present. A double-barreled pH-selective microelectrode (the conventional barrel was used as the reference) was introduced into the droplet to record pHᵢ evolution. In this experiment series, S was 51–58 mV (pH of the testing solutions was 6.8–7.8). Reported pHᵢ values were obtained after 20 min.

Data analysis. Results are given as means ± SE. The significance of the results was assessed by Student’s unpaired t-test. P < 0.05 was considered significant.

RESULTS

Effect of NH₄⁺ on ⁸⁶Rb uptake. In this experimental series, ⁸⁶Rb uptake was measured in β₂- and in α₁-β₂-expressing oocytes in the presence of 200 µM KCl and of inhibitors of endogenous membrane K⁺ transport systems (as detailed in METHODS). Functional expression of the colonic H⁺-K⁺-ATPase dramatically increased ouabain-sensitive ⁸⁶Rb uptake compared with the expression of β₂ alone (Fig. 1, A and B). The addition of 1–85 mM NH₄Cl to assay solutions significantly reduced ⁸⁶Rb uptake by α₁-β₂-expressing oocytes (Fig. 1A). NH₄Cl did not affect ⁸⁶Rb uptake by β₂-expressing oocytes (Fig. 1B). These results are consistent with an NH₄Cl-induced effect on the ionic transport mediated by the colonic H⁺-K⁺-ATPase.

The above results do not indicate whether NH₄Cl acts directly on the pump or if the pump is affected by other effects of NH₄Cl on the oocyte. In oocytes, the extracellular addition of NH₄Cl is known to induce...
Effects of NH₄Cl and trimethylamine-HCl on membrane potential and intracellular pH

86Rb uptake by transport pathways [10 µM ouabain, 5 mM barium, 10 mM NH₄Cl (or 10 mM trimethylamine-HCl)]. Significance of results was assessed by Student's unpaired t-test. *P < 0.05.

Table 1. Effects of NH₄Cl and trimethylamine-HCl on membrane potential and intracellular pH in β₁- and α₁β₁-expressing oocytes

<table>
<thead>
<tr>
<th>Subunit Expressed</th>
<th>Vm, mV</th>
<th>pHᵢ</th>
<th>n</th>
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<tbody>
<tr>
<td>NH₄Cl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β₁</td>
<td>−22.7 ± 2.2</td>
<td>−14.8 ± 1.7</td>
<td>7.7 ± 1.3</td>
</tr>
<tr>
<td>α₁β₁</td>
<td>−18.1 ± 1.0</td>
<td>−6.2 ± 0.9</td>
<td>11.9 ± 1.3*</td>
</tr>
<tr>
<td>Trimethylamine-HCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β₁</td>
<td>−21.8 ± 1.2</td>
<td>−14 ± 0.8</td>
<td>7.2 ± 1.5</td>
</tr>
<tr>
<td>α₁β₁</td>
<td>−15.3 ± 2.4</td>
<td>−9 ± 2.5</td>
<td>6.3 ± 0.3*</td>
</tr>
</tbody>
</table>

Results are means ± SE; n = no. of oocytes from 2–4 independent experiments. Membrane potential (Vm, mV) and intracellular pH (pHᵢ) were measured with double-barreled (conventional and pH selective) microelectrodes. Oocytes were superfused with a 200 µM KCl-containing Ringer solution (plus 10 µM ouabain, 5 mM barium, 10 mM NH₄Cl (or 10 mM trimethylamine-HCl)), then by a similar solution containing an amine (10 mM NH₄Cl or 10 mM trimethylamine-HCl). Significance of results (vs. amine-induced ∆Vm, and ∆pHᵢ for β₁-expressing oocytes) was assessed by Student's unpaired t-test. *P < 0.05. †Not significant.
NH$_4^+$ may act as a functional substrate of the colonic H$^+$.K$^+$-ATPase. The reduction in $^{86}$Rb uptake observed in the presence of 200 µM KCl [i.e., a concentration below the potassium $K_m$ of the colonic H$^+$.K$^+$-ATPase (10)] and of NH$_4$Cl (from the 1 mM concentration) suggests that NH$_4^+$ may be either a competitive substrate for the colonic H$^+$.K$^+$-ATPase, or a noncompetitive inhibitor of the pump. We addressed this question with the following experiments.

In the first approach, we analyzed the rate of initial $\Delta$pH$_i$, induced by 10 mM NH$_4$Cl in the presence of 200 µM KCl; as discussed in DISCUSSION, the slope of pHi, as a function of time (dpHi/dt) can be considered to be a reflection of the initial NH$_4^+$ influx into the oocyte. If NH$_4$Cl inhibited the colonic H$^+$.K$^+$-ATPase, dpHi/dt values in both $\alpha_1\beta_1$-expressing oocytes and $\beta_1$-expressing oocytes should be similar because they would reflect NH$_4^+$ influx via residual endogenous NH$_4^+$ pathways (uninhibited by 10 µM ouabain, 5 mM BaCl$_2$, 10$^{-5}$ M bumetanide, and 1 mM DPC). If NH$_4$Cl entered the cell via the colonic H$^+$.K$^+$-ATPase, dpHi/dt should be enhanced in $\alpha_1\beta_1$-expressing oocytes, reflecting NH$_4^+$ influx by residual endogenous NH$_4^+$ pathways plus NH$_4^+$ influx via the colonic H$^+$.K$^+$-ATPase. In $\beta_1$-expressing oocytes, dpHi/dt measured in the presence of NH$_4$Cl was 0.054 ± 0.002 pH units (U$_{pH}$/min) (n = 9). In $\alpha_1\beta_1$-expressing oocytes, NH$_4$Cl-induced dpHi/dt was significantly enhanced (0.097 ± 0.004 U$_{pH}$/min; n = 9; P < 0.001). When $\alpha_1\beta_1$-expressing oocytes were exposed to 2 mM ouabain, NH$_4$Cl-induced dpHi/dt was 0.051 ± 0.004 U$_{pH}$/min (n = 7), similar to that observed in $\beta_1$-expressing oocytes (P > 0.4). These results are consistent with NH$_4^+$ influx into the oocyte via the colonic H$^+$.K$^+$-ATPase. As a control test, it was observed that trimethylamine-HCl (10 mM) induced similar dpHi/dt values in $\beta_1$- and in $\alpha_1\beta_1$-expressing oocytes [0.051 ± 0.002 U$_{pH}$/min (n = 6) vs. 0.047 ± 0.001 U$_{pH}$/min (n = 3); P = 0.1], consistent with the absence of effect of this amine$^{86}$Rb uptake.

Next, we tested the capacity of $\alpha_1\beta_1$-expressing oocytes to acidify their extracellular medium in the absence of extracellular KCl. As shown in Table 2, the lack of this extracellular substrate prevented the extracellular acidification previously reported (10). However, when 10 mM NH$_4$Cl was added, $\alpha_1\beta_1$-expressing oocytes acidified the KCl-free medium, which is opposite to the effect observed for $\beta_1$-expressing oocytes. This last observation excluded the possibility that NH$_4$Cl may cause extracellular acidification by a mechanism unrelated to colonic H$^+$.K$^+$-ATPase function. Consistent with this conclusion was the finding that adding 2 mM ouabain to the NH$_4$Cl-containing KCl-free medium prevented the pH$_o$ decrease caused by $\alpha_1\beta_1$-expressing oocytes. These results are consistent with NH$_4^+$ being able to replace K$^+$ on the colonic H$^+$.K$^+$-ATPase.

To further support this conclusion, oocytes were incubated for 2 days in KCl-free Ringer solution (supplemented with 10 µM ouabain, 10$^{-5}$ M bumetanide, 5 mM BaCl$_2$, and 1 mM DPC) before [Na$^+$]; was measured. The absence of K$^+$ should lead to colonic H$^+$.K$^+$-ATPase inhibition. Consequently, [Na$^+$]; values should be the same in $\alpha_1\beta_1$- and $\beta_1$-expressing oocytes, because colonic H$^+$.K$^+$-ATPase-mediated Na$^+$ extrusion from cytosol should be blocked (9). [Na$^+$]; was measured for oocytes superfused with the KCl-free solution (composition as above) or with a similar solution containing 1 or 10 mM NH$_4$Cl. Results are summarized in Fig. 3. In the absence of NH$_4$Cl, [Na$^+$]; values were indeed similar in $\beta_1$-expressing and in $\alpha_1\beta_1$-expressing oocytes. The presence of NH$_4$Cl in the superfusate did not alter [Na$^+$]; in $\beta_1$-expressing oocytes, whereas it significantly decreased [Na$^+$]; in $\alpha_1\beta_1$-expressing oocytes, except when 2 mM ouabain was added. This confirms that NH$_4^+$ can replace K$^+$ on the colonic H$^+$.K$^+$-ATPase. Figure 3, inset, shows that, with a 1 or 10 mM concentration of external KCl, [Na$^+$]; is slightly lower in $\alpha_1\beta_1$-expressing oocytes than with 1 or 10 mM NH$_4$Cl, a finding that is consistent with a higher pump affinity for K$^+$ than for NH$_4^+$.

Table 2. Effect of NH$_4$Cl on the extracellular pH of $\beta_1$- and $\alpha_1\beta_1$-expressing oocytes

<table>
<thead>
<tr>
<th>pH$_o$</th>
<th>$\beta_1$</th>
<th>$\alpha_1\beta_1$</th>
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<tr>
<td></td>
<td>0 mM NH$_4$Cl</td>
<td>10 mM NH$_4$Cl</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.55 ± 0.01$^*$</td>
<td>7.57 ± 0.01$^*$</td>
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</table>

Results, obtained after 20 min, are means ± SE; n = no. of oocytes from 3 separate experiments. Extracellular pH (pH$_o$) was measured with pH-selective microelectrodes in a KCl-free, weakly buffered (initial pH = 7.6) droplet (containing 10 µM ouabain, 5 mM barium, 10$^{-5}$ M bumetanide, and 1 mM DPC) surrounding the oocytes. The droplet sometimes contained 10 mM NH$_4$Cl and 2 mM ouabain. Significance of results (compared to initial pH of droplet) was assessed by Student's paired t-test. * P < 0.05. † Not significant.
cells, because of the similarity between NH$_4^+$ and K$^+$ hydrated radii (21). Abundant literature concerns NH$_4^+$ transport by the renal Na$^+$-K$^+$-2Cl$^-$ cotransporter and Na$^+$-K$^+$-ATPase (for reviews, see Refs. 16, 21, and 32). However, very few studies have been devoted to possible NH$_4^+$ transport by the renal H$^+$-K$^+$-ATPases. It was reported that NH$_2^+$ stimulates Sch-28080-sensitive and ouabain-insensitive ATP hydrolysis in the rat outer medullary collecting duct (OMDC) (15). In that study, NH$_4^+$ transport was not specifically demonstrated, but the pharmacological profile of NH$_4^+$-supported ATP

Apparent affinity of the colonic H$^+$-K$^+$-ATPase for NH$_4^+$ was determined in the absence of NH$_4^+$ or in presence of NH$_4^+$ concentrations (30), and was used to determine the apparent inhibition constant for NH$_4^+$ (K$_i$). From this experiment and three others, K$_i$ was found to be 6.3 ± 0.8 mM.

**DISCUSSION**

It is well known that NH$_4^+$ may substitute for K$^+$ in numerous membrane transport systems of various

![Graph](image)

**Fig. 3.** Effect of extracellular NH$_4$Cl on intracellular Na$^+$ activity ([Na$^+$]) in $\alpha$- and $\alpha$-$\beta$-expressing oocytes. Intracellular Na$^+$-selective microelectrodes were used to measure [Na$^+$] in $\beta$-expressing (solid bars) and in $\alpha$-$\beta$-expressing (open bars) oocytes. Oocytes were bathed by a K$^+$-free medium (with 10 µM ouabain, 5 mM barium, 10 $^{-5}$ M bumetanide, and 1 mM DPC), sometimes supplemented with 1 or 10 mM NH$_4$Cl, as indicated below bars. Hatched bar, [Na$^+$] in $\alpha$-$\beta$-expressing oocytes exposed to 10 mM NH$_4$Cl-2 mM ouabain. Results are means ± SE; n = 3–8 oocytes from 3 independent experiments. Significance of results is indicated with respect to [Na$^+$] measured in $\beta$-expressing oocytes. NS, not significant. *P < 0.05. Inset: to compare effects of extracellular NH$_4$Cl and extracellular KCl (1 or 10 mM, as indicated below bars) on [Na$^+$], [Na$^+$] was also measured in oocytes bathed by a NH$_4$Cl-free medium (in presence of 10 µM ouabain, 5 mM barium, 10 $^{-5}$ M bumetanide, and 1 mM DPC), sometimes supplemented with 1 or 10 mM KCl. Results are means ± SE; n = 3–8 oocytes. Significance of results is indicated with respect to [Na$^+$] measured in $\beta$-expressing oocytes. *P < 0.05.

![Graph](image)

**Fig. 4.** Representative experiment of kinetics of $^{86}$Rb uptake by $\alpha$-$\beta$-expressing oocytes measured in presence of increasing NH$_4^+$ concentrations. A: plot of 1/V vs. 1/[K$^+$] (V = velocity; [K$^+$] = K$^+$ concn = 0.2, 1, and 5 mM) in absence of NH$_4^+$ (○) or in presence of 1.5, 5, or 10 mM NH$_4^+$ (●). Results are means from 5–8 oocytes. K$_m$ for K$^+$ is 666 µM in absence of NH$_4^+$ and increases with increasing [NH$_4^+$], whereas maximal velocity (V$_{max}$) is unchanged. B: plot of slopes of reciprocal plots (shown in A) against [NH$_4$Cl], fitted by linear regression (r = 0.987). Intercept on abscissa gives $-K_i = -7.66$ mM where K$_i$ is inhibitor constant.
hydrolysis was consistent with that of the gastric H⁺-K⁺-ATPase (24) and not with that of the colonic H⁺-K⁺-ATPase [which is Sch-28080 insensitive and ouabain sensitive (10)]. The colon, from which colonic H⁺-K⁺-ATPase was cloned (11), is the major site of NH₄⁺ reabsorption, but, to our knowledge, the involvement of this pump in this transport has not been investigated. Therefore, to determine whether the colonic H⁺-K⁺-ATPase could mediate NH₄⁺ transport, we studied the effects of NH₄Cl on ⁸⁶Rb uptake, on pHᵢ, and on [Na⁺]ᵢ after functional expression in Xenopus oocytes of the renal α₁β₁ association, i.e., the catalytic αᵣ subunit together with the β₁ subunit of the Na⁺-K⁺-ATPase (7). Our results are consistent with NH₄⁺ transport mediated by the colonic H⁺-K⁺-ATPase.

In our study, difficulties arising from the presence of endogenous NH₄⁺ pathways were circumvented 1) by performing the experiments in the presence of inhibitors of the NH₄⁺ transport systems so far identified in the oocyte (8), 2) by using β₁-expressing oocytes as controls, and 3) by inhibiting the colonic H⁺-K⁺-ATPase with millimolar concentrations of ouabain (10). We conclude that the significant ⁸⁶Rb uptake decrease by α₁β₁-expressing oocytes caused by NH₄Cl (Fig. 1A) did not reflect an effect of NH₄Cl on the ionic transport mediated by endogenous K⁺ pathways and that α₁β₁ expression did not induce the overexpression or activation of endogenous K⁺- and NH₄Cl-stimulated transport systems because 1) in the presence of NH₄Cl, β₁-expressing oocytes did not show a change in ⁸⁶Rb uptake (Fig. 1B) and 2) in the presence of 2 mM ouabain, the ⁸⁶Rb uptake by α₁β₁-expressing oocytes was unaffected by NH₄Cl (Fig. 1A, inset). Finally, a major effect of ∆Vᵢₐₙ and ∆pHᵢ on the function of the expressed colonic H⁺-K⁺-ATPase was ruled out because trimethylamine-HCl did not alter ⁸⁶Rb uptake; this lack of effect also indicates that amines have no general effect on the pump. Taken together, these results are consistent with a direct effect of NH₄⁺ on the colonic H⁺-K⁺-ATPase.

Results of functional experiments performed in the presence of a low K⁺ concentration or in K⁺-free medium are consistent with the acceptance of NH₄⁺ as a substrate for the colonic H⁺-K⁺-ATPase. The pHᵢ decrease, observed with no external K⁺ and with NH₄Cl, is consistent with H⁺ extrusion by the colonic H⁺-K⁺-ATPase: of the excreted protons, some combined with NH₃ but enough remained free to decrease the droplet pH. The pHᵢ experimental series indicated that NH₄⁺ may substitute for K⁺ and also that expression of α₁β₁ subunits leads to an NH₄⁺/H⁺ exchange in the absence of external K⁺. The decrease in [Na⁺]ᵢ under K⁺-free conditions [with endogenous Na⁺-K⁺-ATPase inhibited by micromolar concentrations of ouabain (6)] suggests that the colonic H⁺-K⁺-ATPase can also extrude Na⁺, as it does in the presence of external K⁺ (9). We therefore conclude that the exchange of Na⁺ and H⁺ with either NH₄⁺ or K⁺ is mediated by the colonic H⁺-K⁺-ATPase. The initial rate of change of pHᵢ points to a colonic H⁺-K⁺-ATPase-mediated NH₄⁺ influx. Because of the intracellular buffering power, ω, the proton-equivalent flux is only partly reflected by dpHᵢ/dt. Nonetheless, even if ω were lower in α₁β₁-expressing oocytes than in control oocytes (because of a high pHᵢ), several observations support the view that enhancement of dpHᵢ/dt is a reliable index of an increased NH₄⁺ flux. First, in the presence of 2 mM ouabain, the NH₄Cl-induced dpHᵢ/dt in α₁β₁-expressing oocytes is similar to that in control oocytes, despite their different pHᵢ values (7.28 ± 0.03, n = 3, vs. 7.66 ± 0.004, n = 7). Second, trimethylamine-HCl induced similar dpHᵢ/dt values in α₁β₁-expressing oocytes and in control oocytes (here again, pHᵢ values were different). Third, this compound induced a significantly lower dpHᵢ/dt than did NH₄Cl in α₁β₁-expressing oocytes (here, pHᵢ values were the same). Thus these results support the interpretation that enhanced NH₄Cl-induced dpHᵢ/dt reflected an increased NH₄⁺ influx, unless the H⁺-extrusion rate was lower in α₁β₁-expressing oocytes than in control oocytes. The latter possibility is unlikely, because the differences between the inward Na⁺ chemical gradients and the outward H⁺ chemical gradients (thus the driving force for Na⁺/H⁺ exchange) in α₁β₁- and β₁-expressing oocytes (47 vs. 42 mM; calculated from [Na⁺]ᵢ and pHᵢ measurements) are not different; moreover, functional expression of H⁺-K⁺-ATPase would, if anything, enhance H⁺ extrusion. Taken together, these results therefore support the conclusion that NH₄⁺ is transported into the cell by the colonic H⁺-K⁺-ATPase at a higher rate than that at which protons are extruded, resulting in dpHᵢ/dt enhancement. The simplest explanation for a proton-equivalent influx overwhelming the proton efflux is the concomitant Na⁺ efflux by the colonic H⁺-K⁺-ATPase (9). It could also be that the colonic H⁺-K⁺-ATPase is also electrogenic, carrying net positive charge(s) into the cell: this condition would also lead to a proton-equivalent influx exceeding the proton efflux.

In agreement with functional studies, kinetic data presented in Fig. 4 indicate that NH₄⁺ acts as a competitive inhibitor of K⁺. The apparent Kᵢ for NH₄⁺ was estimated to be ~6.5 mM. This value appears to be higher than the Kᵢ for K⁺, which is below 1 mM (Ref. 10 and the present study; see Fig. 4A), but both values were obtained by measuring the uptake of ⁸⁶Rb, which is a K⁺ surrogate. As previously indicated, NH₄⁺ was reported to stimulate an Sch-28080-sensitive K⁺-ATPase activity in rat OMDC (15) with a rather high affinity constant, 2 mM. However, from that study and the present one, it cannot be concluded that the gastric H⁺-K⁺-ATPase has a higher affinity for NH₄⁺ than the colonic H⁺-K⁺-ATPase, because the two studies use different techniques and different cell systems. By measuring ⁸⁶Rb fluxes mediated by a ouabain-sensitive K⁺ transport system in inner medullary cells of the rat, an apparent Kᵢ for NH₄⁺ of 11 mM was reported (33). In that study, it was proposed that the ouabain-sensitive system that mediates NH₄⁺ entry into the cell was the Na⁺-K⁺-ATPase, NH₄⁺ being transported from the interstitium into the cytosol. Whereas colonic H⁺-K⁺-ATPase was considered to be mostly expressed in
OMDC (23, 29), a recent study reported its presence in the inner medullary collecting duct (IMCD) (26). Thus whether the ouabain-sensitive, NH$_4^+$-transporting system reported by Wall and Koger (33) represents only the Na$^+$-K$^+$-ATPase or might also represent the colonic H$^+$-K$^+$-ATPase is not yet clear. Our results shed light on the possible transport of NH$_4^+$ by the colonic H$^+$-K$^+$-ATPase in the medullary collecting duct and in the colon. In the medullary collecting duct, luminal NH$_4^+$ is secreted mainly by means of H$^+$ secretion in parallel with NH$_3$ diffusion (see Ref. 13 for a review). The apical location of the colonic H$^+$-K$^+$-ATPase is supported by immunolocalization (using polyclonal antibodies) in OMCD (29) and by a recent functional study of IMDC (26). Apical reabsorption of NH$_4^+$ in place of K$^+$ by the colonic H$^+$-K$^+$-ATPase would imply that this pump mediates NH$_4^+$ transport against a net NH$_4^+$ secretion. In the thick ascending limb of Henle’s loop, in which net NH$_4^+$ reabsorption occurs, a negative feedback regulation of NH$_4^+$ absorption was proposed to account for the apical Na$^+$-H$^+$(NH$_4^+$) exchanger (3). By analogy, it could be proposed that the entry of NH$_4^+$ into the cell from the lumen via an apically located colonic H$^+$-K$^+$-ATPase may be a feedback mechanism of net NH$_4^+$ excretion during hypokalemia, a condition that induces colonic H$^+$-K$^+$-ATPase overexpression (1, 12, 18, 23, 26). Hypokalemia increases NH$_4^+$ production by the proximal tubule and increases NH$_4^+$ reabsorption in the thick ascending limb of Henle’s loop, finally resulting in increased urinary excretion of NH$_4^+$ (21). Thus hypokalemia-induced colonic H$^+$-K$^+$-ATPase expression may limit metabolic alkalosis, which is partly the result of increased NH$_4^+$ excretion.

In the colon from which it was cloned (11), the colonic H$^+$-K$^+$-ATPase is localized on the luminal side (29). The lumen of the colon is bathed by a very high ammonia (NH$_3$ plus NH$_4^+$) concentration; the small part absorbed accounts for almost all circulating ammonia. The mechanisms of ammonia absorption by the colon are of pathophysiological interest (because of the deleterious effects of a high ammonia concentration in blood), but have not been clearly identified (27). It was recently reported that the luminal side of colonic crypts acts as a permeation barrier for either NH$_3$ or NH$_4^+$, a finding which is consistent with NH$_4^+$ absorption via the paracellular pathway or via cells other than the crypt cells (31); as a matter of fact, it was previously concluded that the apical membrane of surface cells of rat colon is endowed with large NH$_4^+$ permeation (22). Because the colonic H$^+$-K$^+$-ATPase is present in surface cells but not in crypt cells (18), we suggest that further work should focus on the involvement of colonic H$^+$-K$^+$-ATPase in colonic NH$_4^+$ absorption.

In conclusion, our findings indicate that NH$_4^+$ competes with K$^+$ for an external site of colonic H$^+$-K$^+$-ATPase, when it is expressed in Xenopus oocytes. The transport of NH$_4^+$ by colonic H$^+$-K$^+$-ATPase in various cells and its physiological relevance remain to be established, and the ionic transport mode(s) of this cationic pump has to be further determined.

We thank M. S. Crowson and G. E. Shull for donating the full-length cDNA coding for the α-subunit of the colonic P-ATPase and J. B. Lingrel for kindly providing the rat Na$^+$-K$^+$-ATPase β$_2$-subunit cDNA. We thank T. Anagnostopoulos for continuous encouragement throughout this study, S. R. Thomas for helpful comments, and P. Hulin for technical assistance. M. Cougnon was supported by a fellowship from the Fondation pour la Recherche Médicale, and P. Bouyer was supported by a fellowship from the Association pour la Recherche contre le Cancer.

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Received 1 February 1999; accepted in final form 18 May 1999.

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