Role of PDK1 in Regulation of Gastric Acid Secretion

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Key Words

PI3 kinase • Stomach • K^+/H^+ ATPase • K^+ recycling
• H^+ secretion

Abstract

Pharmacological inhibition of phosphoinositide 3-kinase (PI3K) has previously been shown to enhance basal gastric acid secretion. Signaling of PI-3-kinase includes activation of the phosphoinositide dependent kinase PDK1. We thus hypothesized that PDK1 may influence gastric acid secretion. In the present study gastric acid secretion in mice expressing ~20 % of PDK1 (*pdk1^{hm}*) was compared to gastric acid secretion in their wild type littermates (*pdk1^{wt}*). According to BCECF-fluorescence cytosolic pH in isolated gastric glands was similar in *pdk1^{hm}* and in *pdk1^{wt}* mice. Na^+ -independent pH recovery ($\Delta pH/min$) following an ammonium pulse, which reflects K^+/H^+ -ATPase activity, was however, significantly faster in *pdk1^{hm}* than in *pdk1^{wt}* mice. In both genotypes, $\Delta pH/min$ was abolished in the presence of K^+/H^+ -ATPase inhibitor omeprazole (100 μM). Increase of local K^+ -concentrations to 35 mM (replacing $Na^+/NMDG$) significantly increased $\Delta pH/min$ in both, *pdk1^{hm}* and *pdk1^{wt}* mice, and abrogated the differences between

genotypes. Similarly, treatment with 5 μM forskolin as well as stimulation of protein kinase C with phorbol ester phorbol 12 myristate 13 acetate (100 nM) enhanced $\Delta pH/min$ to almost identical values in *pdk1^{hm}* and in *pdk1^{wt}* mice. Protein kinase A inhibitor H89 (50 nM) decreased $\Delta pH/min$ in *pdk1^{hm}* to values similar to those in *pdk1^{wt}*. In conclusion, deficient activity of PDK1 leads to a marked increase of gastric acid secretion. The present observations thus disclose a novel element in the regulation of gastric H^+ secretion.

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Introduction

Gastric acid secretion is accomplished by the H^+/K^+ ATPase, which secretes H^+ in exchange of K^+ [1-4]. Availability of luminal K^+ is ascertained by recirculation of K^+ through luminal KCNQ1/KCNE K^+ channels [5-7]. Genetic knockout of KCNQ1 abrogates gastric acid secretion, a finding highlighting the significance of this channel in the regulation of gastric acid secretion [8]. Gastric acid secretion is stimulated by cAMP [2, 4, 9],

Table 1. Gastric acid secretion in *pdk1^{hm}* and *pdk1^{wt}* mice under basal and stimulated conditions. * indicates significant difference between *pdk1^{hm}* and *pdk1^{wt}* mice. # indicates significant difference between respective treatment and control. § indicates significant difference between respective treatment and sham.

		Rate of pH recovery (Δ pH units/ minute)	Intracellular pH (units)	Buffer capacity (mM/pH unit)	Number of mice	Number of cells
Control	Pdk1 ^{wt}	0.037 $\pm$ 0.005	7.16 $\pm$ 0.03	42.11 $\pm$ 5.83	15	156
(Untreated)	Pdk1 ^{hm}	0.090 $\pm$ 0.008*	7.19 $\pm$ 0.02	37.93 $\pm$ 5.51	16	181
Sham	Pdk1 ^{wt}	0.030 $\pm$ 0.009	7.15 $\pm$ 0.03	39.69 $\pm$ 4.75	5	41
(0.1 %DMSO)	Pdk1 ^{hm}	0.099 $\pm$ 0.027*	7.18 $\pm$ 0.06	36.75 $\pm$ 6.51	4	72
Omeprazole	Pdk1 ^{wt}	0.009 $\pm$ 0.004#	7.19 $\pm$ 0.04	31.83 $\pm$ 2.22	3	30
	Pdk1 ^{hm}	0.011 $\pm$ 0.005#	7.15 $\pm$ 0.02	37.25 $\pm$ 18.60	3	50
35 mM K ⁺	Pdk1 ^{wt}	0.296 $\pm$ 0.032#	7.21 $\pm$ 0.05	30.73 $\pm$ 3.60	6	98
	Pdk1 ^{hm}	0.227 $\pm$ 0.025#	7.25 $\pm$ 0.02	35.33 $\pm$ 4.42	6	63
Forskolin	Pdk1 ^{wt}	0.159 $\pm$ 0.021§	7.28 $\pm$ 0.03 §	31.95 $\pm$ 3.74	6	65
	Pdk1 ^{hm}	0.153 $\pm$ 0.026#	7.25 $\pm$ 0.03	35.24 $\pm$ 4.02	6	71
H89	Pdk1 ^{wt}	0.028 $\pm$ 0.008	7.16 $\pm$ 0.06	44.65 $\pm$ 8.85	5	61
	Pdk1 ^{hm}	0.044 $\pm$ 0.013#	7.10 $\pm$ 0.01#	49.79 $\pm$ 6.81	5	40
Carbachol	Pdk1 ^{wt}	0.237 $\pm$ 0.046#	7.30 $\pm$ 0.08#	36.15 $\pm$ 8.26	5	57
	Pdk1 ^{hm}	0.266 $\pm$ 0.050#	7.27 $\pm$ 0.06	54.12 $\pm$ 12.83	5	49
Phorbol ester	Pdk1 ^{wt}	0.209 $\pm$ 0.045§	7.24 $\pm$ 0.06	41.60 $\pm$ 7.96	5	61
	Pdk1 ^{hm}	0.224 $\pm$ 0.014§	7.31 $\pm$ 0.03§	31.70 $\pm$ 5.50	5	51

which is in turn known to stimulate KCNQ/KCNE channels [10-13].

Recent observations suggested the participation of the phosphoinositide 3-kinase (PI3K) in the regulation of gastric acid secretion [14]. Inhibition of PI3K in isolated gastric glands and parietal cells with LY294002 was followed by a substantial increase of gastric acid secretion [14]. The inhibitor was effective through increase of cAMP [14]. Downstream signaling of PI3K includes phosphoinositide-dependent kinase PDK1 and subsequent activation of the SGK and PKB/Akt isoforms [15-21]. As a matter of fact LY294002 similarly blocked the phosphorylation of PKB/Akt [14].

In view of those observations, we hypothesized that PDK1 participates in the regulation of gastric acid secretion. To test this hypothesis, experiments have been performed in gene targeted mice. As the PDK1 knockout mouse is not viable [22], we analyzed the PDK1 hypomorphic mouse (*pdk1^{hm}*) expressing only some 20 % of PDK1 activity compared to the wild type littermates (*pdk1^{wt}*) [22].

Materials and Methods

Animals

PDK1 hypomorphic mice were obtained as described previously [22]. The mice were kindly provided by D. Alessi. The

mice were of mixed (C57/Bl-6/sv129j) background. Sex and age matched mice with age more than 3 months were used for experiments. All animal experiments were conducted according to the guidelines of the American Physiological Society and were approved by local authorities according to the German law for the care and use of laboratory animals.

Treatments

Mice had free access to a standard mouse diet (C1310, Altromin, Heidenau, Germany) and tap water. Effects of omeprazole (100 μ M), phorbol ester (phorbol 12 myristate 13 acetate, TPA) (100 nM), forskolin (5 μ M), carbachol (100 μ M) and H89 (50 nM) were determined by adding the substances directly to the perfusing solutions at the indicated final concentrations. The substances were obtained from Sigma (Deisenhofen, Germany). The stock solutions of omeprazole, phorbol ester and forskolin were prepared in DMSO whereas carbachol and H89 were prepared in standard HEPES buffer. Sham experiments were performed by adding DMSO to the perfusing solution at a final dilution of 0.1 %. Omeprazole was added into the luminal side of the parietal cells while incubating with BCECF. The protons released from cells activate the drug by converting it into its active sulfenic acid/sulfenamide derivative which is further taken up into the cell and eventually binds covalently to H⁺/K⁺ ATPase resulting in the irreversible inhibition of the pump.

Gastric H⁺ secretion

For isolation of gastric glands animals were fasted for 16 hours prior to experiments on wire grids with free access to tap water. After sacrificing the animals the stomach was removed and cut longitudinally. After washing with standard Hepes

Fig. 1. pH recovery in parietal cells following an ammonium pulse in the presence and absence of omeprazole. Alterations of cytosolic pH (Δ pH) in parietal cells following an ammonium pulse. To load the cells with H⁺, 20 mM NH₄Cl was added and Na⁺ removed (replaced by NMDG) in a first step (see bars below each original tracing), NH₄Cl removed in a second step, Na⁺ added in a third step and nigericin (Nig.) applied in a fourth step to calibrate each individual experiment. a. Original tracings illustrating alterations of pH in typical experiments in *pdk1^{wt}* mice (left panels) and *pdk1^{hm}* mice (right panels) in the absence (upper panels) and presence (lower panels) of 100 μ M omeprazole. b. Arithmetic means $\pm$ SEM of pH in parietal cells from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars) in the absence (left bars) and presence (right bars) of 100 μ M omeprazole. c. Arithmetic means $\pm$ SEM of Na⁺ independent alterations of cytosolic pH (Δ pH units/min) in parietal cells from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars) in the absence (left bars) and presence (right bars) of 100 μ M omeprazole. * indicates significant difference between *pdk1^{hm}* mice and *pdk1^{wt}* mice, # indicates significant difference between absence (control) and presence of omeprazole. d. Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) in parietal cells from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars) in the absence (left bars) and presence (right bars) of 100 μ M omeprazole.

solution the fundic and pyloric regions were discarded and the gastric corpus was sliced into 0.3 cm² sections. The tissues were transferred onto the cooled stage of a dissecting microscope and individual glands were carefully detached from the gastric wall by snapping of the tissue using sharpened microdissection tweezers. Care was taken not to touch the apical part of the glands. The glands were attached to a glass coverslip precoated with Cell-Tak adhesive (BD Biosciences).

The solutions, flow lines and perfusion chamber were maintained at 37°C by a thermostatically controlled heating system. The volume of the perfusion chamber was 600 μ l and the flow rate was 4 ml/min for all solutions. For digital imaging of cytosolic pH_i isolated individual glands were incubated in a HEPES-buffered Ringer solution containing 10 μ M BCECF-AM (Molecular Probes, Leiden, The Netherlands) for 15 min at 37°C. After loading, the chamber was flushed for 5 min with Ringer

solution to remove any deesterified dye sticking to the outside of the glands. The perfusion chamber was mounted on the stage of an inverted microscope (Zeiss Axiovert 135), which was used in the epifluorescence mode with a 40 x oil immersion objective (Zeiss Neoplan, Germany). BCECF was successively excited at 490/10 and 440/10 nm, and the resultant fluorescent signal was monitored at 535/10 nm using an intensified charge-coupled device camera (Proxitronic, Germany) and specialized computer software (Metafluor, USA). Between 8 and 20 parietal cells were outlined and monitored during the course of the measurements. The results from each cell were averaged and taken for final analysis. Intensity ratio (490/440) data were converted into pH values using the high-K⁺/nigericin calibration technique [23]. To this end the glands were perfused at the end of each experiment for 5 minutes with standard high-K⁺/nigericin (10 μ g/ml) solution (pH 7.0). The intensity ratio data thus

Fig. 2. Influence of enhanced extracellular K^+ concentration on pH recovery in parietal cells. a. Original tracings illustrating alterations of pH at enhanced extracellular K^+ concentration (35 mM) in $pdk1^{wt}$ mice (left panels) and $pdk1^{hm}$ mice (right panels). b. Arithmetic means $\pm$ SEM of pH in parietal cells at enhanced (35 mM) extracellular K^+ concentration in parietal cells from $pdk1^{hm}$ mice (closed bars) and $pdk1^{wt}$ mice (open bars). c. Arithmetic means $\pm$ SEM of Na^+ independent alterations of cytosolic pH (ΔpH units/min) at enhanced (35 mM) extracellular K^+ concentration in parietal cells from $pdk1^{hm}$ mice (closed bars) and $pdk1^{wt}$ mice (open bars). # indicates significant difference between high or low K^+ (control, see Fig. 1) concentration. d. Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) at enhanced (35 mM) extracellular K^+ concentration in parietal cells from $pdk1^{hm}$ mice (closed bars) and $pdk1^{wt}$ mice (open bars).

obtained were converted into pH values using the r_{max} , r_{min} , pK_a values previously generated from calibration experiments performed in isolated gastric glands to generate a standard non-linear curve (pH range 5 to 8.5).

For acid loading, cells were transiently exposed to a solution containing 20 mM NH_4Cl leading to marked initial alkalinization of cytosolic pH (pH_i) due to entry of NH_3 and binding of H^+ to form NH_4^+ [24]. The acidification of cytosolic pH upon removal of ammonia allowed calculating the mean intrinsic buffering power of the cells [24]. Assuming that NH_4^+ and NH_3 are in equilibrium in cytosolic and extracellular fluid and that ammonia leaves the cells as NH_3 : $\beta = \Delta[NH_4^+]_i / \Delta pH_i$, where ΔpH_i is the decrease of cytosolic pH (pH_i) following ammonia removal and $\Delta[NH_4^+]_i$ is the decrease of cytosolic NH_4^+ concentration, which is identical to the concentration of $[NH_4^+]_i$ immediately before the removal of ammonia. The pK for NH_4^+/NH_3 is 8.9 [25] and at an extracellular pH (pH_o) of 7.4 the NH_4^+ concentration in extracellular fluid ($[NH_4^+]_o$) is 19.37 $[20/(1+10^{pH_o-pK})]$. The intracellular NH_4^+ concentration ($[NH_4^+]_i$) was calculated from: $[NH_4^+]_i = 19.37 \cdot 10^{pH_o-pH_i}$.

The calculation of buffer capacity requires that NH_4^+ exits completely. After the initial decline, pH_i indeed showed

little further change in the absence of Na^+ and presence of omeprazole, indicating that there was no relevant further exit of NH_4^+ .

To calculate the $\Delta pH/min$ during re-alkalinization, a manual linear fit was placed over a narrow pH range (pH 6.7 to 6.9) which could be applied to all measured cells.

The solutions were composed of (in mM): standard Hepes: 115 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 NaH_2PO_4 , 10 glucose, 32.2 Hepes; sodium free Hepes: 132.8 NMDG, 3 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 KH_2PO_4 , 32.2 Hepes, 10 mannitol, 10 glucose (for sodium free ammonium chloride 10 mM NMDG and mannitol were replaced with 20 mM NH_4Cl); high K^+ for calibration 105 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 32.2 Hepes, 10 mannitol, 5 μM nigericin. The pH of the solutions was titrated to 7.4 or 7.0 with HCl/NaOH, HCl/NMDG and HCl/KOH, respectively, at 37°C. Where indicated the K^+ concentration was increased at the expense of Na^+ /NMDG to 35 mM.

Serum gastrin

To determine the serum gastrin levels, the animals were fasted overnight for 16 hours with free access to tap water. About 150 μl of blood specimens were drawn into heparinized

Fig. 3. Effects of forskolin on pH recovery in parietal cells **a.** Original tracings illustrating alterations of pH in typical experiments in *pdk1*^{wt} mice (left panels) and *pdk1*^{hm} mice (right panels) in the absence (upper panels) and presence (lower panels) of forskolin (FSK, 5 μ M). **b.** Arithmetic means $\pm$ SEM of cytosolic pH in parietal cells in the absence (left bars) and presence (right bars) of forskolin (FSK, 5 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars). § indicates significant difference between absence (sham) and presence of forskolin. **c.** Arithmetic means $\pm$ SEM of Na⁺ independent alterations of cytosolic pH (Δ pH units/min) in parietal cells following an ammonium pulse in the absence (left bars) and presence (right bars) of forskolin (FSK, 5 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars). * indicates significant difference between *pdk1*^{hm} mice and *pdk1*^{wt} mice, § indicates significant difference between absence (sham) and presence of forskolin. **d.** Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) in parietal cells in the absence (left bars) and presence (right bars) of forskolin (FSK, 5 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars).

hematocrit capillaries by retro-orbital puncture during light anesthesia with diethylether (Roth, Karlsruhe, Germany). Total serum gastrin concentration was determined by radio immuno assay (RIA) kit according to the manufacturer's (DiaSorin, Dietzenbach, Germany) instructions.

Statistics

Intracellular pH, pH recovery and buffer capacity of all parietal cells from one gland were averaged, and the respective mean values were used for further statistical analysis. Data are provided as arithmetic means $\pm$ SEM, *n* represents the number of mice used. All data were tested for significance using Student's *t*-test with Welch's correction or ANOVA (Dunnets test), where applicable, and only results with *P* < 0.05 were considered statistically significant.

Results

As reported previously [26], the PDK1 hypomorphic mice (*pdk1*^{hm}) were smaller (22.5 ± 0.9 g, *n* = 12) than their wild type littermates (*pdk1*^{wt}, b.w. 32.7 ± 1.3 g, *n* = 12). Food and fluid intake were similar in both genotypes (not shown). Similarly, plasma gastrin levels were similar in *pdk1*^{hm} mice (39.5 ± 3.8 pM) and in *pdk1*^{wt} mice (39.9 ± 3.4 pM).

In isolated gastric glands, cytosolic pH, determined by utilizing BCECF-fluorescence, was similar in *pdk1*^{hm} and in *pdk1*^{wt} mice (Table 1, Fig. 1).

Buffer capacity was again similar in *pdk1*^{hm} and in

Fig. 4. Effects of H89 on pH recovery in parietal cells a. Original tracings illustrating alterations of pH in typical experiments in *pdk1^{wt}* mice (left panels) and *pdk1^{hm}* mice (right panels) in the presence of PKA inhibitor H89 (H89, 50 nM). b. Arithmetic means $\pm$ SEM of cytosolic pH in parietal cells in the presence of H89 (50 nM) in gastric glands from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars). # indicates significant difference between absence (control, see Fig. 1) and presence of H89. c. Arithmetic means $\pm$ SEM of Na^+ independent alterations of cytosolic pH (ΔpH units/min) in parietal cells following an ammonium pulse in the presence of H89 (H89, 50 nM) in gastric glands from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars). # indicates significant difference between absence (control, see Fig. 1) and presence of H89. d. Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) in parietal cells in the presence of H89 (50 nM) in gastric glands from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars).

pdk1^{wt} mice (Fig. 1). Thus, a similar exit of H^+ would lead to similar alterations of cytosolic pH in *pdk1^{hm}* and in *pdk1^{wt}* mice. Accordingly, alterations of cytosolic pH reflected the respective H^+ transport rate across the cell membrane.

Na^+ -independent pH recovery following an ammonium pulse ($\Delta\text{pH}/\text{min}$), which reflected K^+/H^+ -ATPase activity, was significantly (~ 2.5 -fold) faster in *pdk1^{hm}* than in *pdk1^{wt}* mice. As shown in Fig. 1, $\Delta\text{pH}/\text{min}$ was in both genotypes virtually abolished by the addition of the K^+/H^+ -ATPase inhibitor omeprazole (100 μM).

An increase of local K^+ -concentrations to 35 mM (replacing Na^+/NMDG) increased $\Delta\text{pH}/\text{min}$ to similar values in *pdk1^{hm}* and *pdk1^{wt}* mice (Fig. 2). The increase of extracellular K^+ thus again abrogated the differences between genotypes.

Treatment of gastric glands with 5 μM forskolin significantly increased $\Delta\text{pH}/\text{min}$ again to values similar in *pdk1^{hm}* mice and in *pdk1^{wt}* mice (Fig. 3). Thus, the significant differences between the genotypes again disappeared by the treatment with forskolin.

The significant difference between the two genotypes was also abrogated by the treatment of the gastric glands with the protein kinase A inhibitor H89 (50 nM). H89 significantly decreased $\Delta\text{pH}/\text{min}$ in *pdk1^{hm}* mice but was without significant effect in *pdk1^{wt}* mice (Fig. 4).

Stimulation of gastric glands from *pdk1^{hm}* and *pdk1^{wt}* mice with carbachol (100 μM) enhanced $\Delta\text{pH}/\text{min}$ in *pdk1^{hm}* mice and in *pdk1^{wt}* mice again resulting in abrogation of the difference between *pdk1^{hm}* and in *pdk1^{wt}* mice (Fig. 5).

Stimulation of protein kinase C with phorbol ester

Fig. 5. Effects of carbachol on pH recovery in parietal cells. **a.** Original tracings illustrating alterations of pH in typical experiments in *pdk1*^{wt} mice (left panels) and *pdk1*^{hm} mice (right panels) in the presence of carbachol (100 μ M). **b.** Arithmetic means $\pm$ SEM of cytosolic pH in parietal cells in the presence of carbachol (100 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars). # indicates significant difference between absence (control, see Fig. 1) and presence of carbachol. **c.** Arithmetic means $\pm$ SEM of Na⁺ independent alterations of cytosolic pH (Δ pH units/min) in parietal cells following an ammonium pulse in the presence of carbachol (100 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars). # indicates significant difference between absence (control, see Fig. 1) and presence of carbachol. **d.** Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) in parietal cells in the presence of carbachol (100 μ M) in gastric glands from *pdk1*^{hm} mice (closed bars) and *pdk1*^{wt} mice (open bars).

phorbol 12 myristate 13 acetate (100 nM) enhanced Δ pH/min in *pdk1*^{hm} mice and *pdk1*^{wt} mice. Following stimulation with phorbol ester, Δ pH/min was again similar in *pdk1*^{hm} and in *pdk1*^{wt} mice (Fig. 6).

Discussion

The present observations provide evidence for an involvement of phosphoinositide dependent kinase 1 (PDK1) in the regulation of basal gastric acid secretion. The present observations underscore the conclusions of the previous study suggesting that gastric acid secretion is suppressed by phosphoinositide 3-kinase (PI3K)

dependent signaling [14]. Moreover, the present finding defines the PI3K dependent pathway involved. PI3K is apparently effective through PDK1.

The present study did not attempt to define the PDK1 dependent signaling. PDK1 activates the serum and glucocorticoid inducible kinase (SGK) or protein kinase B (PKB/Akt) isoforms [15-21, 27]. In the previous study, pharmacological PI3K inhibition disrupted the phosphorylation of PKB/Akt, a finding suggesting that PKB/Akt could have been the downstream target of PI3K signaling [14]. According to previous observations, the basal gastric acid secretion is not significantly altered by genetic knockout of SGK1 [28]. Thus, defective activation of SGK1 hardly accounts for the observed stimulation of

Fig. 6. Effects of phorbol ester on pH recovery in parietal cells. **a.** Original tracings illustrating alterations of pH in typical experiments in *pdk1^{wt}* mice (left panels) and *pdk1^{hm}* mice (right panels) in the presence of phorbol 12 myristate 13 acetate (TPA, 100 nM). **b.** Arithmetic means $\pm$ SEM of cytosolic pH in parietal cells in the presence of phorbol ester (TPA, 100 nM) in gastric glands from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars). § indicates significant difference between absence (sham, see Fig. 3) and presence of phorbol ester. **c.** Arithmetic means $\pm$ SEM of Na^+ independent alterations of cytosolic pH ($\Delta \text{pH units/min}$) in parietal cells following an ammonium pulse in the presence of phorbol ester (TPA, 100 nM) in *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars). § indicates significant difference between absence (sham, see Fig. 3) and presence of phorbol ester. **d.** Arithmetic means $\pm$ SEM of buffer capacity (mM/pH unit) in parietal cells in the presence of phorbol ester (TPA, 100 nM) in gastric glands from *pdk1^{hm}* mice (closed bars) and *pdk1^{wt}* mice (open bars).

basal gastric acid secretion in the PDK1 deficient mouse or following pharmacological PI3K inhibition. The increase of gastric acid secretion following stimulation with dexamethasone was even blunted in the SGK1 knockout mouse, disclosing a participation of SGK1 in the stimulation of gastric acid secretion by glucocorticoids [28]. Accordingly, stimulation of gastric acid secretion by dexamethasone was similarly blunted by inhibition of PI3 kinase [29]. Glucocorticoids further downregulate cyclooxygenase [30, 31], an effect expected to enhance gastric acid secretion [30–33]. Whether or not prostaglandins influence gastric acid secretion through SGK1 has not been tested thus far. Moreover, future

studies will be required to define the SGK or PKB isoform(s) involved in the suppression of gastric acid secretion.

The present observations further demonstrate that increase of extracellular K^+ concentration enhances gastric acid secretion to a greater extent in wild type mice (*pdk1^{wt}*) than in PDK1 hypomorphic mice (*pdk1^{hm}*). Accordingly, the increase of extracellular K^+ concentration abrogated the differences between the genotypes. This observation suggests that PDK1 dependent signaling influences gastric acid secretion by affecting the availability of luminal K^+ for the K^+/H^+ ATPase.

The recycling of K⁺ from the cell into the lumen is accomplished by KCNQ1 channels, which indeed play a decisive role in gastric acid secretion [8, 34, 35]. The channels are activated not only by SGK1 [36-38], but also by protein kinase A [10-13]. According to the previous study PI3K inhibition increased the formation of cAMP, which should lead to enhanced activation of protein kinase A [14]. Accordingly, the protein kinase A inhibitor H89 substantially decreased the gastric acid secretion in *pdk1^{hm}* mice. Conversely, activation of protein kinase A by forskolin stimulated gastric acid secretion. Both, H89 and forskolin virtually abolished the differences of gastric acid secretion between the genotypes, again highlighting the significance of cAMP in the difference of gastric acid secretion between *pdk1^{hm}* and in *pdk1^{wt}* mice. The K⁺ channels are further activated by protein kinase C [39, 40]. Accordingly, phorbol esters again stimulated the gastric acid secretion

and dissipated the differences between genotypes. Protein kinase C is at least partially involved in the stimulation of gastric acid secretion by the Ca²⁺ receptor [41].

In conclusion, basal gastric acid secretion is significantly stronger in *pdk1^{hm}* than in *pdk1^{wt}* mice. Thus, PDK1 exerts an inhibitory action on basal gastric acid secretion.

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