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PI3 Kinase and PDK1 in the Regulation of the Electrogenic Intestinal Dipeptide Transport

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Key Words

Wortmannin • LY294002 • Phosphatidylinositol-3-kinase • Phosphoinositide dependent kinase • Peptide transport

Abstract

The phosphoinositol 3 kinase (PI3K) and the phosphoinositide dependent kinase (PDK1) stimulate the serum and glucocorticoid inducible kinase (SGK) and protein kinase B (PKB/Akt) isoforms, kinases stimulating a variety of transporters. Most recently, SGK1 was shown to stimulate the peptide transporters PepT1 and PepT2, and to mediate the glucocorticoid stimulation of PepT1. Basal electrogenic intestinal peptide transport was, however, not dependent on the presence of SGK1. The present study explored whether basal electrogenic intestinal peptide transport is dependent on PI3K or PDK1. To this end, peptide transport in intestinal segments was determined utilizing Ussing chamber analysis. Cytosolic pH (pH_i) was determined by BCECF fluorescence. The luminal addition of 5 mM dipeptide gly-gly induced a current (Ip) across intestinal segments. Ip was significantly decreased in the presence of PI3 kinase inhibitors Wortmannin (1 μM) or LY294002 (50 μM). Exposure of isolated intestinal cells to 5 mM gly-gly was followed by cytosolic acidification (ΔpH_i), which was significantly blunted by Wortmannin and by LY294002. Both, Ip and ΔpHi were significantly smaller in PDK1 hypomorphic mice (*pdk1*^{fifl}) than in their wild type littermates (*pdk1*^{wt}). In conclusion, PI3K and PDK1 participate in the regulation of basal peptide transport.

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Introduction

Electrogenic transport of di- and tripeptides and selected peptidomimetics, including β-lactam antibiotics and antiviral drugs, is in large part accomplished by the peptide transporters PepT1 (SLC15A1) and PepT2 (SLC15A2) [1-3]. PepT1 and PepT2 were originally discovered in the small intestine and kidney [4-6], but are expressed in a wide variety of cells.

Little is known about the regulation of peptide transport. Experiments utilizing dual electrode voltage clamp in *Xenopus* oocytes disclosed the ability of the serum- and glucocorticoid-inducible kinase SGK1 to stimulate the PepT1 [7] and PepT2 transporter activity

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[8]. SGK1 [9] was originally cloned as a glucocorticoid-inducible gene [10] and was shown to mediate the stimulating effect of dexamethasone on several transport systems including gastric acid secretion [11], intestinal glucose transport [12], intestinal Na^+/H^+ exchanger activity [12-14], and intestinal peptide transport [7]. Notably, lack of SGK1 did not affect the basal peptide transport but abrogated its stimulation by glucocortioids [7].

Similar to the other SGK isoforms and the protein kinase B (PKB/Akt) isoforms, SGK1 is activated by insulin-like growth factor IGF1 and by insulin via phosphatidyl-inositol 3 (PI3) kinase and phosphoinositide-dependent kinase PDK1 [9, 15-17]. SGK1 upregulates a wide variety of channels and transporters [9], an ability partially shared by the SGK isoforms SGK2 and SGK3 and the PKB/Akt isoforms [9]. Lack of PI3 kinase or of PDK1 is expected to impair the function of those kinase isoforms and their ability to regulate transport systems. Accordingly, pharmacological inhibition of PI3K decreased basal intestinal glucose and amino acid transport [18]. Moreover, basal intestinal Na^+/H^+ exchanger activity [19] and basal intestinal amino acid transport [20] are significantly decreased in PDK1 hypomorphic (*pdk1*$^{fl/fl}$) mice expressing only some 20% of PDK1 [21].

The present paper thus explored, whether the basal intestinal peptide transport is similarly dependent on PI3K and PDK1. To this end, electrogenic peptide transport was determined by Ussing chamber experiments in intestinal segments and PepT1 dependent H^+ entry into isolated intestinal cells determined utilizing BCECF fluorescence. Experiments were performed in intestinal segments and cells from wild type mice in the absence and presence of PI3 kinase inhibitors as well as in *pdk1*$^{fl/fl}$ and sex and age matched wild type littermates (*pdk1*^{wt}).

Materials and Methods

Generation and basic properties of PDK1 hypomorphic mice were described previously [21]. The mice were genotyped from tail tissue using PDK1 and neo-R-specific primers as described [21]. The mice had free access to standard mouse diet (Altromin, Lage, Germany) and tap water. All animal experiments were conducted according to the German law for the welfare of animals and were approved by the local animal committee.

For analysis of electrogenic intestinal peptide transport, jejunal segments were mounted into a custom made mini-Ussing chamber with an opening of 0.00769 cm^2. Under control conditions the serosal and luminal perfusate contained (in mM): 115 NaCl, 2 KCl, 1 $MgCl_2$, 1.25 $CaCl_2$, 0.4 KH_2PO_4, 1.6 K_2HPO_4, 5 Na pyruvate, 25 $NaHCO_3$ 5 mannitol (pH 7.4, NaOH). Where indicated, glycyl-glycine (gly-gly) (5 mM) was added to the luminal perfusate at the expense of mannitol.

In all Ussing chamber experiments the transepithelial potential difference (Vt) was determined continuously and transepithelial apparent resistance (Rt) was estimated from the voltage deflections (ΔVt) elicited by imposing test currents (It). The resulting Rt was calculated according to Ohm's law. The absolute value of the apparent resistance, but not the transepithelial voltage was influenced by the chamber size, i.e. it was higher at larger Ussing chambers. Moreover, the absolute currents were smaller in larger Ussing chambers. Thus, all experiments were made in paired comparisons utilizing the same chamber.

For isolation of intestinal villi animals were fasted for 6 hours prior to the experiments. After sacrificing the animals the proximal 2 cm of the intestine were removed and cut longitudinally. After washing with standard Hepes solution the intestine was sliced into 0.3 cm^2 sections. The tissues were transferred onto the cooled stage of a dissecting microscope and individual villi were detached from the intestine by snapping off the ileal base using sharpened microdissection tweezers. Care was taken not to touch the apical part of the villi. The villi were attached to glass coverslips precoated with Cell-Tak adhesive (BD Biosciences).

For quantitative digital imaging of pH_i, isolated individual villi were incubated in a Hepes-buffered Ringer solution containing 10 µM 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 villi. The perfusion chamber was mounted on the stage of an inverted microscope (Zeiss Axiovert 135), which was used in the epifluorescence mode with a 40x 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). Individual cells from the brush border of the villi were outlined and monitored during the course of the measurement. Intensity ratio data (490/440) were converted into intracellular pH (pH_i) values using the high K^+/nigericin calibration technique [22].

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 µl and the flow rate was 4 ml/min for all solutions.

As shown earlier [7], the acidification of the cell following the removal of Na^+ and ammonium was the result of NH_3 and not due to the decrease of extracellular Na^+ concentration.

The standard Hepes solution was composed of (in mM) 115 NaCl, 5 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 NaH_2PO_4 10 glucose, 32.2 Hepes; for sodium-free Hepes 132.8 NMDG, 3 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 2 KH_2PO_4, 32 Hepes, 10 mannitol, 10 glucose; in sodium-free ammonium chloride 10 NMDG and 10 mannitol were replaced by 20 mM NH_4Cl; high K^+ for calibration was composed of 105 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 32.2 Hepes, 10 mannitol (5 µM nigericin). The pH of the solutions was titrated

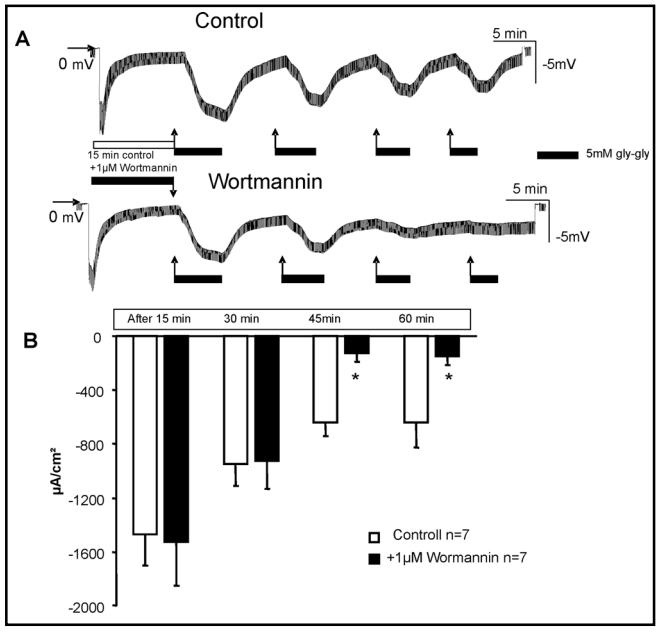


Fig. 1. Wortmannin sensitivity of peptide-induced transepithelial current (Ig) in intestine. Peptide-induced alterations of transepithelial voltage (ΔVt) and peptide-induced current (Ig) in jejunal segments from wild type mice in the absence or presence of Wortmannin (1µM). A: Original tracings from typical experiments illustrating the effect of 5 mM gly-gly (addition indicated by black arrows) on the transepithelial potential difference in the absence (upper panel) and presence (lower panel) of Wortmannin. B: Arithmetic means ± SEM (n = 7) of absolute values of peptide-induced currents in jejunum in the absence (open columns) and presence (closed columns) of 1 µM Wortmannin. * indicates statistically significant difference ($p<0.05$) between the presence and absence of Wortmannin.

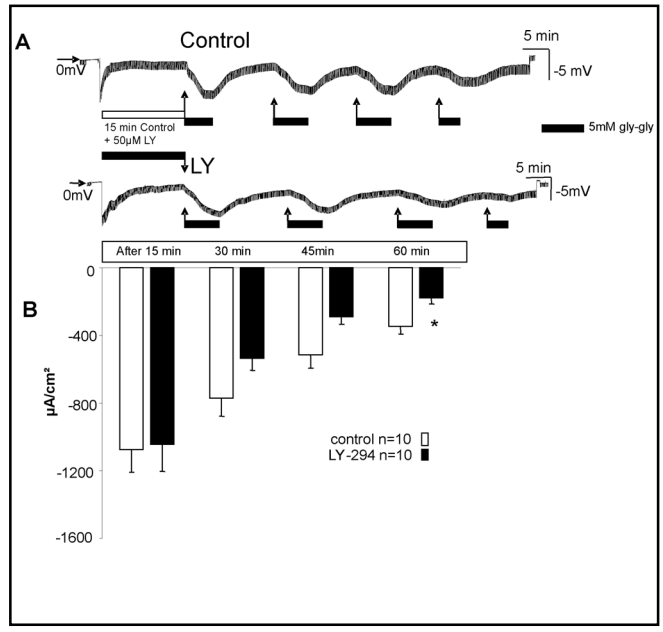


Fig. 2. LY294002 sensitivity of peptide-induced transepithelial current (Ig) in intestine. Peptide-induced alterations of transepithelial voltages (ΔVt) and peptide-induced currents (Ig) in jejunal segments from wild type mice in the absence or presence of LY294002 (50 µM). A: Original tracings from typical experiments illustrating the effect of 5 mM gly-gly (addition indicated by black arrows) on the transepithelial potential difference in the absence (upper panel) and presence (lower panel) of LY294002. B: Arithmetic means ± SEM (n = 10) of absolute values of peptide-induced currents in jejunum in the absence (open columns) and presence (closed columns) of 1 µM LY294002. * indicates statistically significant difference ($p<0.05$) between presence and absence of LY294002.

to 7.4 or 7.0 with HCl/NaOH, HCl/NMDG and HCl/KOH, respectively, at 37°C.

The pH_i was determined prior to and following addition of glycyl-glycine (gly-gly) (5 mM) to the standard Hepes buffer at the expense of sodium chloride. The possibility of simultaneous extrusion of the cytosolic H^+ ion by the membrane Na^+/H^+ exchanger was excluded by treating the tissue with NHE3 specific inhibitor 'dimethylamiloride'. The tissue was treated with dimethylamiloride (20 µM) by adding the substance directly to the incubation medium and to the perfusing solutions at the indicated final concentration. Where indicated, the tissue was treated with wortmannin (1 µM) or with LY294002 (50 µM) for 30 minutes prior to the experiment. Accordingly, sham treatment experiments were performed in the tissues treated with DMSO (0.01 %) for 30 minutes.

The possible untoward toxic effects of wortmannin or LY294002 treatment were studied by performing TUNEL assay on the tissue sections. Briefly, the tissue was cut to 8 µm frozen sections from mouse jejenum and subsequently fixed in 4 % paraformaldehyde for 30 min at room temperature. After rinsing with PBS the samples were permeabilized in a solution of 0.1 % Triton X-100 in sodium citrate for 2 min. Samples, washed with PBS, were then incubated in the TUNEL reaction mix for 1 h at 37°C, according to the manufacturer's instructions (Roche, Germany). Nuclei were stained with DRAQ5™ (Biostatus Limited). Sections were analysed with a confocal laser scanning microscope (Carl Zeiss).

All substances were from Sigma (Taufkirchen, Germany) or Roth (Karlsruhe, Germany).

Data are provided as means ± SEM, *n* represents the number of independent experiments. All data were tested for significance using Student t-test or ANOVA, where applicable, and only results with $p < 0.05$ were considered statistically significant.

Results

A first series of experiments explored the sensitivity of the electrogenic intestinal peptide transport to pharmacological interaction with the PI3 kinase pathway. To this end jejunal segments from wild type mice were

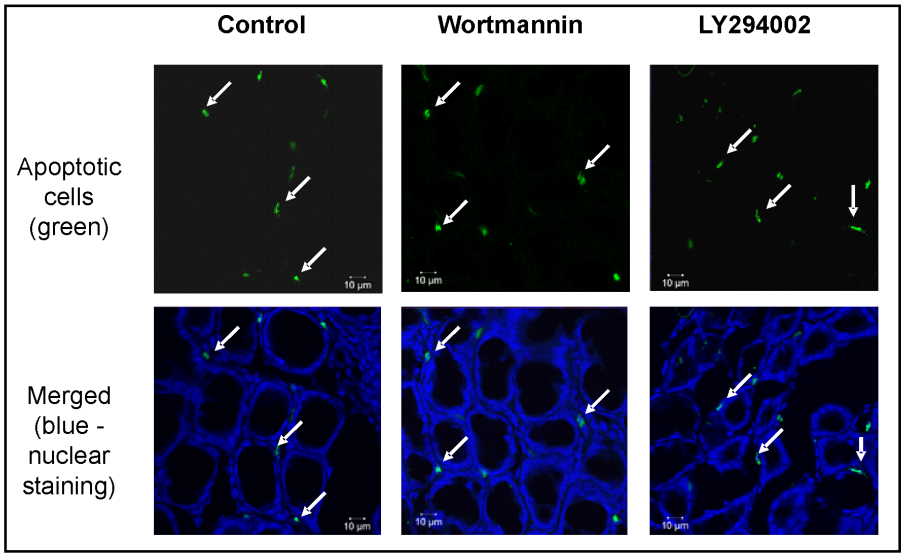


Fig. 3. Apoptosis in jejunum in absence and presence of Wortmannin/ LY294002. TUNEL staining of representative jejunal gut sections. Only few apoptotic cells (arrowheads) were observed in the control tissue (left panels), in the tissue treated with 1 µM Wortmannin for 30 min (middle panels) and in the tissue treated with 50 µM Ly294002 for 30 min (right panels). Fluorescence co-staining for DAPI (blue) and TUNEL-positive nuclei (green) of the same area shown in lower panels. Scale bars = 10 µm.

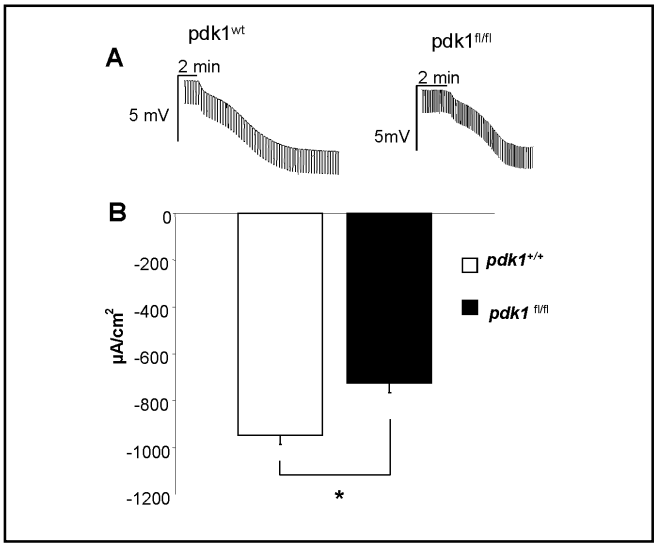


Fig. 4. Impact of PDK1 on peptide-induced intestinal transepithelial current. Peptide-induced alterations of transepithelial voltages (ΔVt) and peptide-induced currents (Ig) in jejunal segments from PDK1 hypomorphic mice (*pdk1*$^{fl/fl}$) and their wild type littermates (*pdk1*^{wt}). A: Original tracings from typical experiments illustrating the effect of 5 mM gly-gly on the transepithelial potential difference in *pdk1*$^{fl/fl}$ (left panel) and *pdk1*^{wt} mice (right panel). B: Arithmetic means ± SEM (n = 7) of peptide-induced currents in jejunum from *pdk1*$^{fl/fl}$ (closed columns) and *pdk1*^{wt} (open columns) mice. * indicates statistically significant difference ($p<0.05$) between *pdk1*$^{fl/fl}$ and *pdk1*^{wt} mice.

mounted into mini-Ussing chambers and electrogenic peptide transport determined utilizing electrophysiological analysis. In the absence of luminal substrates, the transepithelial potential difference (Vt) at the beginning of the experiment amounted to -0.80 ± 0.03 mV (n = 7) and the apparent transepithelial resistance (Rt) to 6.12 ± 0.02 Ω·cm^2 (n = 7) in untreated jejunal segments and to -0.85 ± 0.01 mV (Vt, n = 7) and 6.51 ± 0.05 Ω·cm^2 (Rt, n = 7), respectively, in Wortmannin (1 µM) treated jejunal segments. At the end of the experiment (i.e. after 60 minutes) Vt approached -1.37 ± 0.09 mV (n = 7) and Rt 10.56 ± 0.71 Ω·cm^2 (n = 7) in untreated jejunal segments and -1.58 ± 0.11 mV (Vt, n = 7) and 12.13 ± 0.082 Ω·cm^2 (Rt, n = 7), respectively, in Wortmannin (1 µM) treated jejunal segments.

The addition of the dipeptide glycyl-glycine (gly-gly, 5 mM) replacing mannitol was followed by a lumen-negative shift of the transepithelial potential difference (ΔVt) without a significant change of the apparent transepithelial resistance (Fig. 1). The effect of gly-gly on Vt was reversible upon removal of the dipeptide from the luminal perfusate. ΔVt and Rt allowed the calculation of the peptide-induced current (Ip). Both, ΔVt and Ip declined gradually. The addition of the PI3 kinase inhibitor Wortmannin (1 µM) to the luminal perfusate significantly decreased both ΔVt and Ip (Fig.1).

To consider the theoretical possibility that the current was due to splitting of gly-gly with subsequent electrogenic transport of glycine, additional experiments have been performed elucidating the effect of gly-gly in the presence of saturating glycine concentrations. As a result, the glycine induced currents were similar at 20 mM (-1044_± 146 µA/cm^2, n = 3) and 30 mM (-984 ± 111 µA/cm^2, n = 3) luminal glycine concentration, but was further

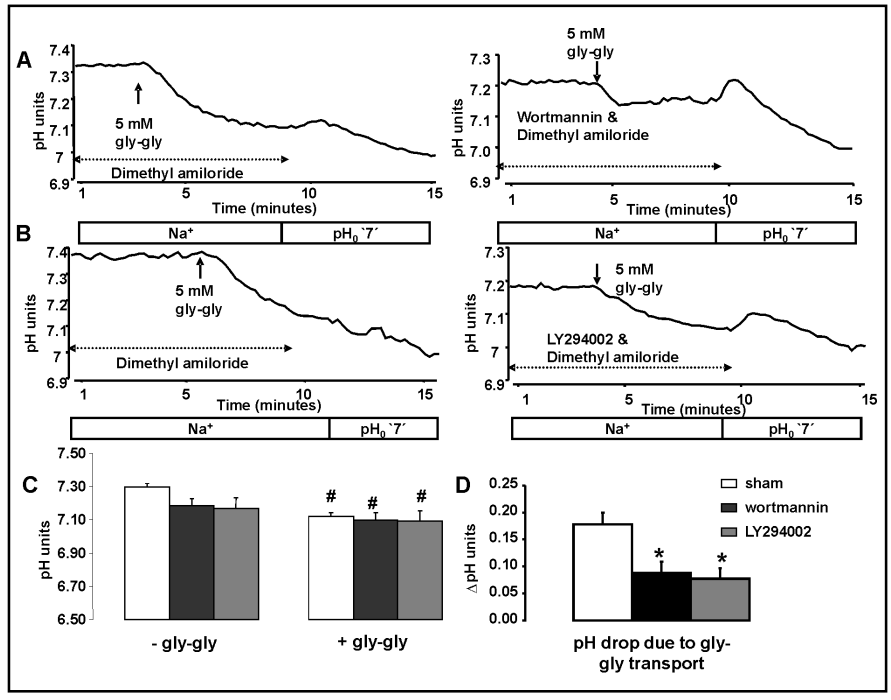


Fig. 5. Sensitivity of peptide-induced cytosolic acidification to PI3 kinase inhibitors. Peptide-induced decrease of cytosolic pH in cells isolated from jejunal segments from wild type mice studied in the presence of dimethylamiloride (20 μM) to inhibit the NHE activity and in the absence or presence of PI3 kinase inhibitors. The cells were superfused with standard HEPES buffer (Na^+) for 2-3 minutes (see bars below each original tracing), then gly-gly was added to the perfusing solution (indicated by arrows) and then perfused with nigericin in a solution buffered to pH 7.0 (pH_0 '7') to calibrate each individual experiment. A. Original tracings from typical experiments illustrating the effect of 5 mM gly-gly (addition indicated by black arrows) on cytosolic pH in the absence (left panel) and presence (right panel) of Wortmannin (1 μM). B. Original tracings from typical experiments illustrating the effect of 5 mM gly-gly (addition indicated by black arrows) on cytosolic pH in the absence (left panel) and presence (right panel) of LY294002 (50 μM). C: Arithmetic means ± SEM (n = 6 - 7 mice in each group) of cytosolic pH prior to addition of (-gly-gly) (left panel) and following addition of (+gly-gly) 5 mM gly-gly (right panel) to sham treated (white bars), Wortmannin treated (grey bars) or LY294002 treated (black bars) intestinal villi. # indicates statistically significant difference ($p<0.05$) to respective initial pH values. D: Arithmetic means ± SEM (n = 6 - 7 mice in each group) of cytosolic acidification ($ÄpH_i$) by addition of 5 mM gly-gly to sham treated (white bars), Wortmannin treated (grey bars) or LY294002 treated (black bars) intestinal villi. * indicates statistically significant difference ($p<0.05$) between sham treatment and wortmannin or LY294002 treatment.

increased by -984 ± 111 μA/cm² (n = 3) following addition of 10 mM gly-gly on top of 20 mM glycine. The gly-gly induced current can thus not be explained by formation of glycine.

Similar observations were made utilizing the more specific PI3 kinase inhibitor LY294002 (50 μM). In the absence of luminal substrates, the transepithelial potential difference (Vt) amounted to -0.82 ± 0.02 mV (n = 10) and the apparent transepithelial resistance (Rt) to 6.31 ± 0.15 Ω·cm² (n = 10) in control jejunal segments and to -0.84 ± 0.03 mV (Vt, n = 10) and 6.36 ± 0.21 Ω·cm² (Rt, n = 10), respectively, in LY294002 treated jejunal segments. Again, the addition of LY294002 (50 μM) to the luminal perfusate significantly blunted both ΔVt and Ip (Fig. 2).

To rule out the possibility that the decline of current was secondary to toxic effects of wortmannin and LY294002, TUNEL assay was performed on the tissues treated with those substances. As seen in Fig. 3, treatment of the tissues with wortmannin or LY294002 did not results in any significant increase in the number of apoptotic cells.

To define the impact of PDK1 on electrogenic intestinal peptide transport, additional experiments were performed in intestinal segments of PDK1 hypomorphic mice *(pdk1^{flfl})* and their wild type littermates *(pdk1^{wt})*. In the absence of luminal substrates, the transepithelial potential difference (Vt) amounted to -0.98 ± 0.06 mV (n = 7) in untreated *pdk1^{flfl}* mice and to -0.91 ± 0.04 mV (n = 7) in untreated *pdk1^{wt}* mice. The apparent transepithelial resistance (Rt) averaged 7.54 ± 0.4 Ω·cm² (n = 7) in *pdk1^{flfl}* mice and 7.01 ± 0.34 Ω·cm² (n = 7) in *pdk1^{wt}* mice. Neither transepithelial potential difference nor apparent transepithelial resistance was significantly different between *pdk1^{flfl}* and *pdk1^{wt}* mice. However, the ΔVt following addition of 5 mM gly-gly was significantly larger in *pdk1^{wt}* than in *pdk1^{flfl}* mice. Accordingly, Ip was significantly larger in *pdk1^{wt}* than in *pdk1^{flfl}* mice (Fig. 4). Thus, PDK1 deficiency impairs electrogenic intestinal peptide transport.

In a second series of experiments, cytosolic pH (pHi) was determined in both, the presence and absence of luminal peptide. As illustrated in Fig. 5, the addition of gly-gly (5 mM) to the luminal perfusate was followed by cytosolic acidification ($ΔpH_i$). The acidification reflects

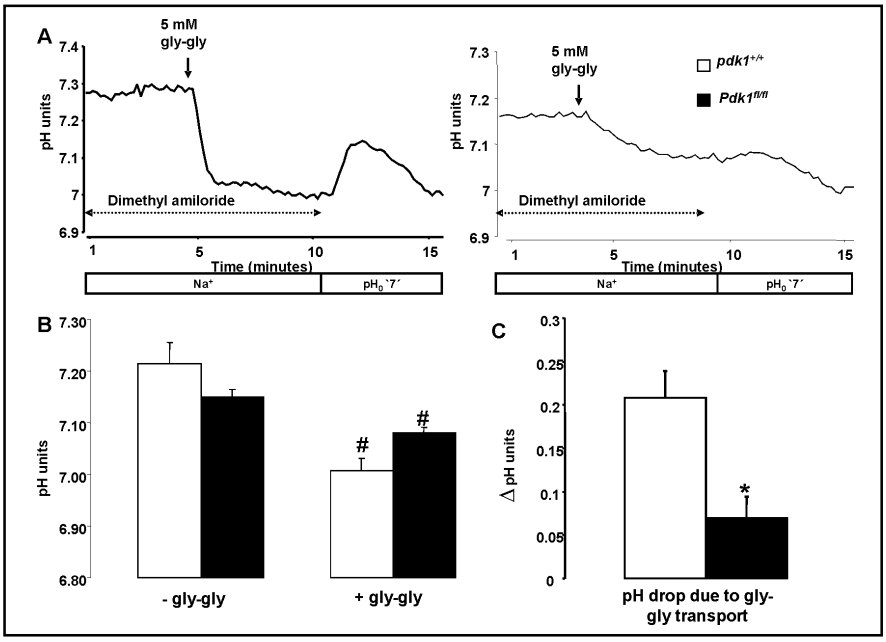


Fig. 6. Impact of PDK1 on peptide-induced cytosolic acidification of intestinal cells. Peptide-induced decrease of cytosolic pH in cells isolated from jejunal segments from PDK1 hypomorphic mice *(pdk1$^{fl/fl}$)* and their wild type littermates *(pdk1^{wt})* in the presence of dimethylamiloride (20 μM). A: Original tracings from typical experiments illustrating the effect of 5 mM gly-gly (addition indicated by black arrows) on cytosolic pH in *pdk1$^{fl/fl}$* (left panel) and *pdk1^{wt}* mice (right panel). B: Arithmetic means ± SEM (n = 4) of cytosolic pH prior to (-gly-gly) and following (+gly-gly) addition of 5 mM gly-gly to intestinal villi from *pdk1$^{fl/fl}$* (black bars) and *pdk1^{wt}* (white bars) mice. # indicates statistically significant difference ($p<0.05$) to respective initial pH values. C: Arithmetic means ± SEM (n = 4) of cytosolic acidification (ΔpH_i) by addition of 5 mM gly-gly to intestinal villi from *pdk1$^{fl/fl}$* (black bars) and *pdk1^{wt}* (white bars) mice. * indicates statistically significant difference ($p<0.05$) between *pdk1$^{fl/fl}$* and *pdk1^{wt}* mice.

H^+ coupled transport of peptide and cannot be explained by degradation of gly-gly to glycine and subsequent Na^+ coupled electrogenic transport of glycine.

The treatment of the villi with Wortmannin (1 μM) did not exert a significant effect on pHi in the absence of luminal peptide but significantly blunted ΔpH_i following addition of 5 mM gly-gly (Fig. 5). Similarly, LY294002 (50 μM) treatment significantly blunted the ΔpHi following addition of gly-gly (Fig. 5).

Further experiments were performed in isolated intestinal cells from *pdk1$^{fl/fl}$* and *pdk1^{wt}* mice. In the absence of gly-gly, the pH_i was not significantly different between *pdk1$^{fl/fl}$* and *pdk1^{wt}* mice. The ΔpH_i following addition of 5 mM gly-gly was, however, significantly larger in *pdk1^{wt}* than in *pdk1$^{fl/fl}$* mice (Fig. 6). Thus, PDK1 deficiency blunted the cytosolic acidification resulting from entry of H^+ into intestinal cells during transport of the dipeptide gly-gly.

Discussion

The present observations reveal the sensitivity of intestinal electrogenic peptide transport to pharmacological knockout of the PI3 kinase and the partial genetic knockout of PDK1. As shown earlier [7], genetic knockout of the serum and glucocorticoid inducible kinase SGK1 did not appreciably compromise the basal activity of the intestinal peptide transporter but blunted its stimulation by glucocorticoids. Thus, a PI3K and PDK1 regulated kinase other than SGK1 presumably accounts for the present observations. The kinases could be SGK2, SGK3 [23] or any of the three protein kinase B (PKB/Akt) isoforms [24-26]. Those kinases share many of the target proteins and mechanisms of transport regulation with SGK1 [9]. Along those lines, electrogenic glucose transport is significantly decreased in the SGK3-deficient mouse in the absence of glucocortioids [27]. The SGK and protein kinase B isoforms could collectively contribute to the stimulating effect of insulin-like growth factor on intestinal nutrient uptake [28-30].

The stimulation of the intestinal peptide transporter by PI3K and PDK1 sensitive signaling is at least in part independent from upregulation of PepT1 transcription, as SGK1 stimulated both peptide transporters PepT1 [7] and PepT2 [8] in the *Xenopus* oocyte expression system, where the carriers are expressed by injection of cRNA and effects of kinases on carrier activity cannot be due to genomic regulation of expression. Instead, the activity of PepT1 and PepT2 may be enhanced by SGK1 and the related kinases through increase of protein trafficking to and/or protein stability within in the cell membrane or by increase of the transporter activity without affecting the carrier protein abundance. The SGK isoforms have

previously been shown to phosphorylate Nedd4-2 leading to decreased interaction of this ubiquitin ligase with carrier proteins in the cell membrane thus delaying the degradation of those carrier proteins [9, 31]. Alternatively, SGK1 enhances trafficking by interaction with the scaffolding protein NHERF2 [8, 9, 32] or by stimulating the phosphatidylinositol-3-phosphate-5-kinase PIKfyve [33]. All those mechanisms enhance the carrier protein abundance in the cell membrane, an effect expected to be less rapid than direct activation of the carrier protein.

Peptide transport leads to the entry of H^+ and thus to cytosolic acidification. The activity of PepT1 and PepT2 is paralleled by Na^+/H^+ exchanger activity, leading to recycling of H^+ ions. The carrier involved is most likely NHE3 [12] and is thus inhibited by the NHE3 inhibitor S3226 [19]. The NHE3 activity prevents undue cytosolic acidification following H^+ coupled transport. The present experiments were performed in the presence of the NHE inhibitor dimethylamiloride to avoid modification of the peptide induced cytosolic acidification by NHE activity. Na^+-independent primary active H^+ extrusion is negligible as compared to Na^+-dependent H^+ extrusion [19]. Accordingly, the recovery of cytosolic pH after an ammonium pulse is negligible prior to readdition of Na^+ [7]. NHE3 is similarly a target of SGK1 dependent regulation [9] and is thus similarly expected to downregulate upon PI3 kinase inhibition. As a matter of fact, cytosolic pH does gradually decline in the presence of PI3 kinase inhibitors.

The dipeptide transport slowly decreases even in the absence of Wortmannin or LY294002, pointing to a gradual decrease of PI3 kinase dependent stimulation of transport. PI3 kinase signaling is not constitutively active but requires stimulation by growth factors or insulin [9]. Presumably, the concentration of growth factors decreases in the isolated tissue by washout leading to the decline of transport activity.

At least in theory, the decline of transport activity could have been due to suicidal cell death of the epithelial cells, which is counteracted by PI3 kinase signalling [34-41]. However, within the experimental period, we could not observe significant increase of suicidal cell death following application of Wortmannin or LY294002.

In conclusion, the basal intestinal peptide transport is blunted by pharmacological inhibition of PI3 kinase and genetic suppression of PDK1. The experiments thus reveal a powerful mechanism in the regulation of intestinal peptide transport.

Acknowledgements

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