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Epinephrine-induced hyperpolarization of pancreatic islet cells is sensitive to PI3K–PDK1 signaling

Ying Zhang [a], Ekaterina Shumilina [a], Hans-Ulrich Häring [b], Florian Lang [a,*], Susanne Ullrich [a,b]

[a] Institute of Physiology, University of Tübingen, Gmelinstrasse 5, D-72076 Tübingen, Germany
[b] Department of Internal Medicine IV, University Hospital Tübingen, Germany

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ABSTRACT

Epinephrine inhibits insulin release by activation of K^+ channels and subsequent hyperpolarization of pancreatic beta cells. The present study explored whether epinephrine-induced hyperpolarization is modified by phosphatidylinositol 3-kinase (PI3K) and phosphatidylinositide-dependent kinase PDK1. Perforated patch-clamp was performed in islet cells isolated from PDK1 hypomorphic mice (*pdk1*$^{fl/fl}$), expressing only 20% of PDK1, and in their wild-type littermates. At 16.8 mM glucose, the cell membrane was hyperpolarized by epinephrine (1 μM), an effect significantly blunted in *pdk1*$^{fl/fl}$ and abrogated in wild-type cells by inhibition of PI3K with wortmannin (100 nM) or LY294002 (10 μM). The hyperpolarizing effect of epinephrine in pancreatic islet cells is thus sensitive to PI3K and PDK1.

1. Introduction

Insulin release from pancreatic islet cells is inhibited by epinephrine via activation of α_2-adrenoceptors [1,2]. Epinephrine is at least partially effective through activation of K^+ channels, subsequent hyperpolarization of the cell membrane, inhibition of voltage gated Ca^{2+} channels and decrease of cytosolic Ca^{2+} activity [2–4]. The signaling linking receptor activation to altered channel activity has, however, remained incompletely understood.

Effects of epinephrine in other cell types have been shown to involve phosphatidylinositol 3-kinase (PI3K) [5,6]. PI3K dependent signaling includes the phosphoinositide-dependent kinase PDK1 [7–13].

The present study explored whether the effect of epinephrine on cell membrane potential is modified by inhibition of the PI3K with the unrelated PI3K inhibitors (LY294002) 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one [14,15] and wortmannin [16]. To define the contribution of PDK1, gene targeted mice have been analyzed. As PDK1 knockout mice are not viable [17], experiments have been performed in PDK1 hypomorphic mice (*pdk1*$^{fl/fl}$) expressing only some 20% of PDK1 activity compared to their wild-type littermates (*pdk1*$^{+/+}$) [17].

Abbreviations: PKB/Akt, protein kinase B; FCS, fetal calf serum; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; LY294002, PI3K inhibitor; PI3K, phosphatidylinositol 3-kinase

* Corresponding author. Fax: +49 7071 29 5618.
E-mail address: florian.lang@uni-tuebingen.de (F. Lang).

2. Materials and methods

Phosphoinositide-dependent kinase-1 hypomorphic mice (*pdk1*$^{fl/fl}$) and *pdk1*$^{+/+}$ littermates with mixed C57/Bl-6/sv129j background, 3–6 months old and sex and age matched were kindly provided by D. Alessi (Biocenter, Dundee, GB) [17]. The *pdk1*$^{fl/fl}$ mice express 10–25% of PDK1 and are viable while mice completely lacking functional PDK1 are not viable [17]. For the experiments shown in Fig. 1 wild-type mice of C57/BL6 background were used. All animal experiments were conducted according to the German law for the care and use of laboratory animals and were approved by local authorities.

For culture of pancreatic islet cells, the mice were killed by inhalation of CO_2. Digestion of pancreatic tissue was initiated by retroverse injection of 3 ml collagenase solution containing 1 mg/ml collagenase (Serva, Heidelberg, Germany) into the bile duct. The pancreas was cut out from other adjacent organs and tissues. Collagenase digestion was performed for 10 min at 37 °C in a water bath and terminated by addition of 30 ml ice-cold Hank's solution. Thereafter the pancreatic fragments were sedimented by centrifugation (150 g for 1 min at RT). White islets were isolated from exocrine tissue under a dissection microscope by hand selection.

The purified islets were resuspended in sterile 10 ml ice-cold PBS (Ca^{2+}/Mg^{2+}-free, pH 7.2, Gibco) and a single cell preparation obtained by digestion of islets with trypsin (0.025% in PBS) as described previously [18]. Isolated islet cells were resuspended in culture medium at a concentration of 50 islets/100 μl and

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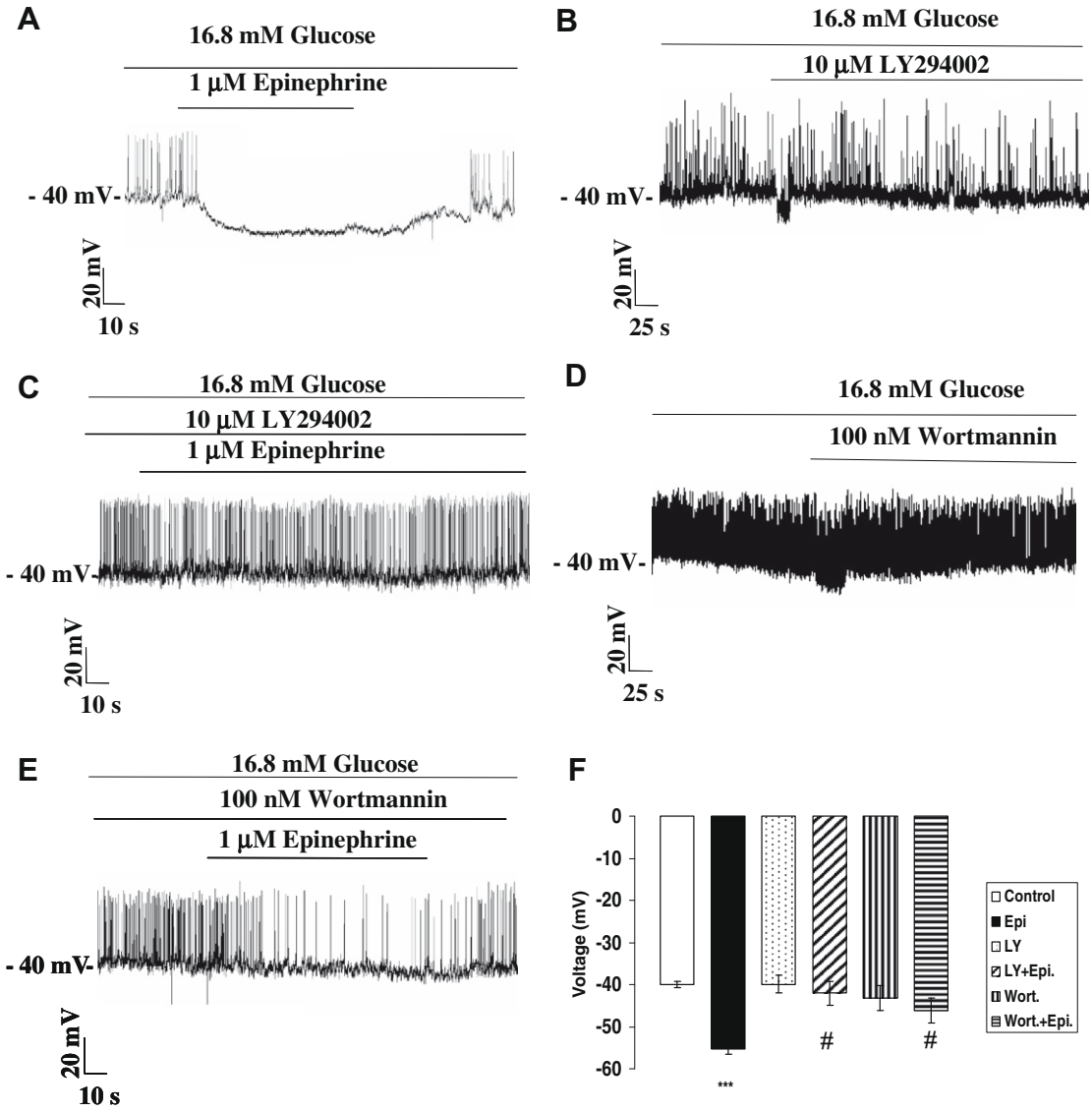

Fig. 1. PI3K inhibitors counteract the hyperpolarizing effect of epinephrine on the islet cell membrane. (A) Original tracing illustrating the cell membrane potential in an islet cell from a wild-type mouse of C57/BL6 background. The experiments have been performed prior to and following an increase of extracellular glucose concentration to 16.8 mM, subsequent addition and then washing-out of 1 μM epinephrine. (B) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM and subsequent addition of LY294002 (10 μM). (C) Original tracing of the cell membrane potential following an addition of first LY294002 (10 μM) and then epinephrine (1 μM). (D) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM and subsequent addition of wortmannin (100 nM). (E) Original tracing of the cell membrane potential following an addition of first wortmannin (100 nM) and then epinephrine (1 μM). (F) Cell membrane potential (±S.E.M., $n = 7–16$) at 16.8 mM glucose concentration measured before (control, white bars) and after application of either epinephrine (1 μM), or LY294002 (10 μM) or wortmannin (100 nM) as well as after application of epinephrine on top of either LY294002 or wortmannin. *** indicates significant difference from control from epinephrine (ANOVA, $P < 0.001$).

20–25 μl drops were placed into culture dishes. The cells were grown in RPMI 1640 supplemented with 10% (v/v) fetal calf serum (FCS), 10 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 1 mM Na-pyruvate, 2 mM L-glutamine, 50 i.u. penicillin/ml and 50 μg streptomycin/ml. About 8–10 dishes were prepared from 100 islets. Cells were kept in an incubator 1–3 days before the experiments.

Epinephrine, LY294002 hydrochloride and wortmannin (purchased from Sigma–Aldrich, Deisenhofen, Germany) were applied at the indicated concentrations to the bath solution. The substances were prepared as aliquots and stored in −20 °C up to 2 months before use.

For patch-clamp experiments, the culture dish with attached primary mouse islet cells was mounted onto the stage of an inverted microscope. The volume of the recording chamber was 0.2–0.3 ml. The bath solution containing in mM 145 NaCl, 5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 HEPES and 5.6 glucose (pH 7.4) was superfused into the chamber at a rate of 5–6 ml/min. The experiments were carried out at room temperature (20 °C). Experiments were performed using perforated whole-cell current clamp recording to monitor membrane potential by use of an EPC9 patch-clamp amplifier (HEKA Electronic Company, Germany). Patch pipettes were pulled from borosilicate glass capillaries (GC150TF-7.5, 1.5 mm O.D. × 1.17 mm I.D. Harvard Apparatus Ltd., UK) and heat-polished using a DMZ universal puller (Zeitz, Augsburg, Germany). Pipettes had a resistance of 4.0–6.0 MΩ when filled with the internal solution containing in mM: 95 K-Gluconate, 30 KCl, 1 $MgCl_2$, 3 EGTA, 1 $CaCl_2$, 1.2 NaH_2PO_4 and 4.8 Na_2HPO_4 (pH 7.3). The pipette solution contained amphotericin B (1 mg/ml pipette solution prepared just before use and protected from light). After

formation of a cell-attached configuration, perforated whole-cell configuration was formed within 5–10 min. Only stable voltage measurements were used for analysis, i.e. when the access resistance was stable and lower than 20 MΩ.

Data are presented as means ± S.E.M. for the indicated number of cells examined. ANOVA and Student's paired *t*-test were used for statistical analysis, as appropriate. *P* values <0.05 were accepted to indicate statistical significance.

3. Results

In control islet cells from wild-type mice the increase of extracellular glucose concentration from 5.6 mM to 16.8 mM significantly ($P < 0.001$) depolarized the cell membrane (from −55.16 ± 1.15 mV at 5.6 mM glucose to −38.82 ± 1.07 mV within 62.6 ± 6.9 s following an increase to 16.8 mM ($n = 38$)) and triggered action potentials reflecting opening of Ca^{2+} channels (Fig. 1A). About 22% of the islet cells did not respond with cell membrane depolarization and appearance of action potentials upon increasing glucose concentration in the bath. Those cells were not used for the following experiment. Most probably, 78% cells represent beta cells, whereas the other 22% represent other types of islet cells. The distribution of the different islet cell types may be variable depending on culture conditions [19]. Application of epinephrine (1 μM) in the continued presence of 16.8 mM glucose resulted in a significant ($P < 0.001$) hyperpolarization of the cell membrane to −55.2 ± 1.4 mV ($n = 16$; Fig. 1A and F). The hyperpolarizing effect of epinephrine was observed in each of the 16 recordings performed, started approximately 10–15 s after its application and reached a maximal value within some 20–30 s. After removal of epinephrine the membrane potential reversed within 1–5 min in 12 out of 16 recordings completely to a potential of −38.6 ± 2.0 mV ($n = 12$, Fig. 1A).

Application of the LY294002 (10 μM; $n = 7$) prior to epinephrine did not significantly ($P = 0.086$) modify the cell membrane potential (Fig. 1B) but prevented the epinephrine-induced hyperpolarization (LY294002 alone −40.0 ± 2.1 mV vs. LY294002 plus epinephrine −42.1 ± 2.8 mV; $n = 7$ respectively; Fig. 1C and F). Application of wortmannin, another inhibitor of PI3K (100 nM) was similarly without significant effect on the membrane potential in the absence of epinephrine (Fig. 1D), but prevented the epinephrine-induced hyperpolarization (wortmannin −45.7 ± 3.6 mV vs. wortmannin plus epinephrine −48.8 ± 3.7 mV; $n = 7$; $P = 0.08$; Fig. 1E and F). In the presence of wortmannin, but not of LY294002, epinephrine reduced the action potential frequency (Fig. 1E). The frequency was 1.8 ± 0.4 action potentials/s in the presence of wortmannin and 0.5 ± 0.1 action potentials/s in the presence of wortmannin + epinephrine (analyzed utilizing 70–80 s recordings in four independent experiments; $P < 0.05$). In experiments, when the cells were first treated with epinephrine to induce hyperpolarization, application of LY294002 or wortmannin resulted in subsequent depolarization and reappearance of action potentials (Fig. 2). In the presence of epinephrine the membrane potential increased from −46.7 ± 3.7 mV to −38.7 ± 3.6 mV after addition of LY294002 and to −32.1 ± 3.5 mV following addition of wortmannin ($n = 3$, $P < 0.05$).

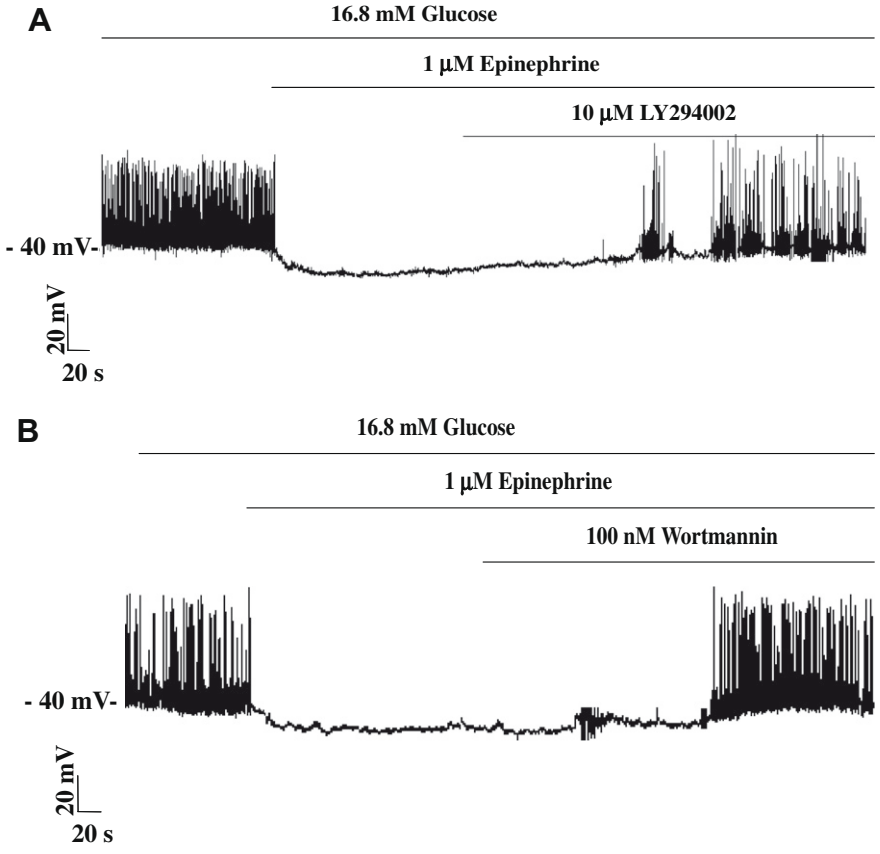

Fig. 2. PI3K inhibitors lead to recovery of the islet cell membrane potential after epinephrine action. (A) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM, subsequent addition of 1 μM epinephrine and then application of LY294002 (10 μM) on top of epinephrine obtained from an islet cell from a wild-type mouse of C57/BL6 background. (B) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM, subsequent addition of 1 μM epinephrine and then application of wortmannin (100 nM) on top of epinephrine obtained from an islet cell from a wild-type mouse of C57/BL6 background.

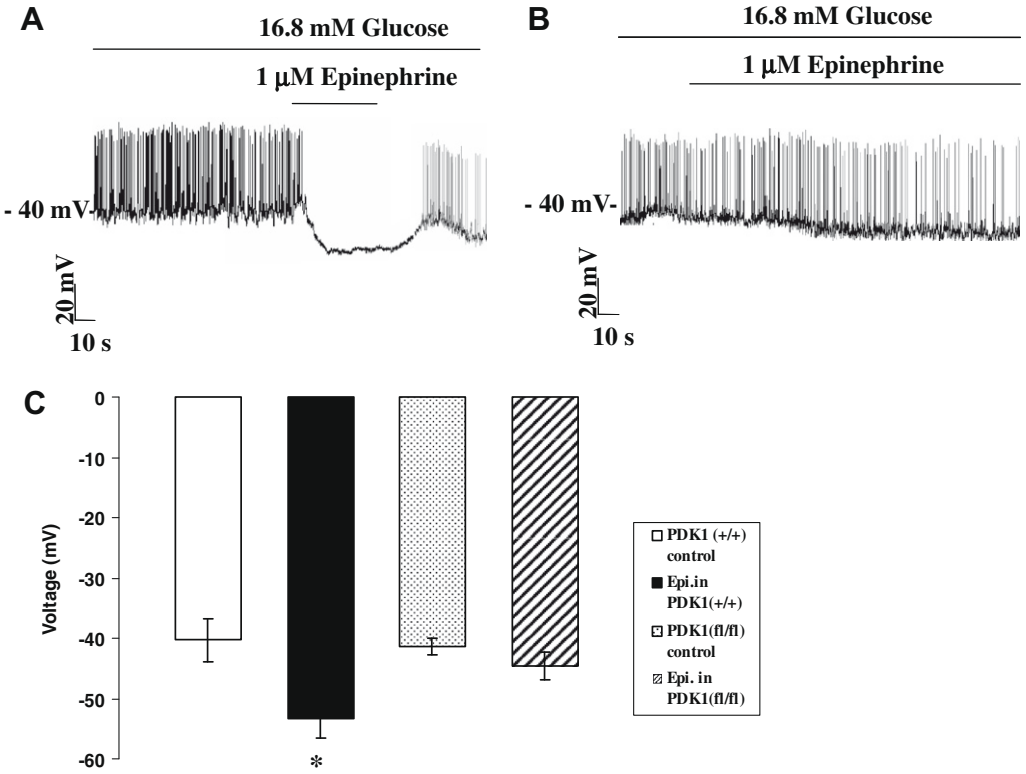

Fig. 3. Effect of epinephrine on the cell membrane potential in islet cells from PDK1 wild-type and hypomorphic mice. (A) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM, subsequent addition and then washing-out of 1 μM epinephrine in an islet cell from a PDK1 wild-type mouse (*pdk1*$^{+/+}$, C57/Bl-6/sv129j background). (B) Original tracing of the cell membrane potential following an increase of extracellular glucose concentration to 16.8 mM and subsequent addition of 1 μM epinephrine in an islet cell from a PDK1 hypomorphic mouse (*pdk1*$^{fl/fl}$). (C) Cell membrane potential (±S.E.M., $n = 5–7$ cells from three mice in each group) at 16.8 mM glucose concentration in the absence or in the presence of 1 μM epinephrine in islet cells from either *pdk1*$^{+/+}$ or *pdk1*$^{fl/fl}$ mice. * indicates significant difference from control *pdk1*$^{+/+}$ (ANOVA, $P \leqslant 0.05$).

The hyperpolarizing effect of epinephrine was also observed in *pdk1*$^{+/+}$ mouse islet cells (Fig. 3A and C) but was abrogated in islet cells from PDK1 hypomorphic mice (*pdk1*$^{fl/fl}$) (Fig. 3B). The exposure of the cells to 16.8 mM glucose and 1 μM epinephrine resulted in a significant ($P < 0.05$) hyperpolarization from −40.3 ± 3.6 mV to −53.4 ± 3.2 mV ($n = 5$ cells from three mice) in *pdk1*$^{+/+}$ mouse islet cells (Fig. 3A and C). In contrast, as shown in Fig. 3B and C the application of epinephrine (1 μM) in the presence of 16.8 mM glucose did not significantly ($P = 0.11$) hyperpolarize *pdk1*$^{fl/fl}$ mouse islet cells (−41.5 ± 1.4 mV prior to and −44.6 ± 2.3 mV following epinephrine, $n = 7$ cells from three mice).

4. Discussion

The present study demonstrates that the hyperpolarizing effect of epinephrine in pancreatic islet cells is abrogated by two unrelated inhibitors of the PI3K and is significantly blunted in mice with decreased expression of PDK1. Effects of LY294002 on K^+ channels have been demonstrated in insulin secreting cells at a concentration of 50 μM, in the present study LY294002 was used at a five times lower concentration and the similar inhibitory effect of wortmannin and the blunted hyperpolarizing effect of epinephrine in PDK1 hypomorphic mice strongly suggest a mechanism dependent on PI3K and PDK1 signaling [20]. As a matter of fact, PI3K and PDK1 have previously been shown to upregulate voltage gated K^+ channels [10]. The reduction of action potential frequency by epinephrine in the presence of wortmannin and in PDK1 deficient cells suggests that epinephrine not only activates K^+ channels but may also inhibit Ca^{2+} channels independent of PI3K activity. An inhibition of those channels has been observed in several cells including pancreatic beta cells [21].

Earlier studies revealed that in mice epinephrine-induced hyperpolarization is not due to opening of K_{ATP} channels [22]. This observation was confirmed using mouse islet cells deficient in functional K_{ATP} channels [4]. A study with specific K^+ channel inhibitors suggested that epinephrine activated inwardly rectifying potassium (GIRK) channels that are sensitive to high concentrations of tertiapin-Q [23]. In insulin secreting cells from rat and hamster, epinephrine seems to act through the activation of K_{ATP} channels, as tolbutamide counteracted the hyperpolarizing effect of epinephrine [24,25]. The opening of K_{ATP} channels by leptin has been shown to depend on PI3K signaling that is dependent on PTEN and actin reorganisation [26,27].

Since the epinephrine induced hyperpolarization is mimicked by specific alpha$_2$ adrenoceptor agonists and abolished by pertussis toxin pretreatment, the effect is obviously mediated by alpha$_2$ adrenoceptor activation coupled to G_i/G_o proteins [2,25,28,29]. In other cell types, involvement of PI3K in the signaling of α-adrenergic receptors and G proteins has been demonstrated [6,30,31]. As wortmannin has been shown to deplete cellular PIP_2 pools [32] and GIRK channel activity depends on PIP_2, it seems likely that epinephrine is unable to open GIRK channels in cells depleted of PIP_2 due to treatment with wortmannin. Whether epinephrine activates GIRK channels by increasing PIP_2 or through another mechanism remains to be elucidated [33,34].

Epinephrine and somatostatin have been found to inhibit glucose-dependent augmentation of insulin mRNA [35]. This observation may be explained by the recent finding that epinephrine

inhibits glucose-mediated stimulation of ERK1/2 in insulin secreting cells, an effect of epinephrine which is sensitive to pertussis toxin [36].

The present study did not define the signaling pathway downstream of PDK1. PI3K/PDK1 dependent signaling involves protein kinase B (PKB/Akt) and SGK isoforms [11,13,37–39]. Both SGK [38] and PKB/Akt [40] have previously been shown to regulate K^+ channels. Moreover, SGK1 has previously been shown to account for the upregulation of Kv1.5 K^+ channels in pancreatic beta cells by dexamethasone [18]. Without stimulation by dexamethasone, the expression level of SGK1 is, however, low in pancreatic beta cells [18] and thus, SGK1 probably does not account for the observed PI3K/PDK1 dependent hyperpolarization. The possibility must be kept in mind that PDK1 may be active without PI3K signaling and that pharmacological inhibition of PI3K may thus not completely disrupt PDK1 dependent signaling.

Galanin and somatostatin, similar to epinephrine, inhibit insulin release, whereby they apparently utilize a distinct receptor but a similar mechanism [3]. Future experiments may reveal whether or not galanin and somatostatin similarly act though activation of the PI3K pathway. However, it should be noted that inhibition of insulin secretion by epinephrine but also by somatostatin may imply mechanisms unrelated to ion channel activity [41]. The present paper does not rule out PI3K dependence or independence of those additional adrenergic effects.

The PDK1 deficiency of the $pdk1^{fl/fl}$ mouse does not only affect the electrophysiology of the pancreatic islet cells but impairs insulin dependent regulation of PKB, S6K and RSK [17]. Moreover, intestinal and renal electrogenic glucose transport is significantly decreased in those mice [42]. The alterations of plasma glucose concentration following an intrapertitoneal glucose load are, however, not significantly different between $pdk1^{+/+}$ and $pdk1^{fl/fl}$ mice [42].

In conclusion, the epinephrine induced hyperpolarization of pancreatic islet cells depends on the activation of PI3K and PDK1. The present observations thus disclose a novel mechanism in the regulation of pancreatic K^+ channels which may be important not only for the hyperpolarizing effects of epinephrine but for electrophysiological effects of other hormones and growth factors known to signal through the PI3K pathway.

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