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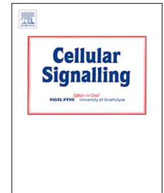

Inositol pyrophosphates and Akt/PKB: Is the pancreatic β-cell the exception to the rule?

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ABSTRACT

The inositol pyrophosphate, diphosphoinositol pentakisphosphate (IP_7), is thought to negatively regulate the critical insulin signaling protein Akt/PKB. Knockdown of the IP_7-generating inositol hexakisphosphate kinase 1 (IP6K1) results in a concomitant increase in signaling through Akt/PKB in most cell types so far examined. Total *in vivo* knockout of IP6K1 is associated with a phenotype resistant to high-fat diet, due to enhanced Akt/PKB signaling in classic insulin regulated tissues, counteracting insulin resistance. In contrast, we have shown an important positive role for IP6K1 in insulin exocytosis in the pancreatic β-cell. These cells also possess functional insulin receptors and the feedback loop following insulin secretion is a key aspect of their normal function. Thus we examined the effect of silencing IP6K1 on the activation of Akt/PKB in β-cells. Silencing reduced the glucose-stimulated increase in Akt/PKB phosphorylation on T308 and S473. These effects were reproduced with the selective pan-IP6K inhibitor TNP. The likely explanation for IP_7 reduction *decreasing* rather than *increasing* Akt/PKB phosphorylation is that IP_7 is responsible for generating the insulin signal, which is the main source of Akt/PKB activation. In agreement, insulin receptor activation was compromised in TNP treated cells. To test whether the mechanism of IP_7 inhibition of Akt/PKB still exists in β-cells, we treated them at basal glucose with an insulin concentration equivalent to that reached during glucose stimulation. TNP potentiated the Akt/PKB phosphorylation of T308 induced by exogenous insulin. Thus, the IP_7 regulation of β-cell Akt/PKB is determined by two opposing forces, direct inhibition of Akt/PKB versus indirect stimulation via secreted insulin. The latter mechanism is dominant, masking the inhibitory effect. Consequently, pharmacological strategies to knock down IP6K activity might not have the same positive output in the β-cell as in other insulin regulated tissues.

1. Introduction

Inositol pyrophosphates are now considered to be key cellular regulators with roles in signal transduction and in a broad spectrum of critical cellular processes, including vesicle trafficking and exocytosis, apoptosis and cell proliferation, telomere length and cytoskeletal regulation as well as polyphosphate homeostasis [1–8]. Some of these processes may be driven by action of the inositol pyrophosphate, 5-diphosphoinositol pentakisphosphate (5-PPIP5 or IP_7) on the key signaling protein Akt/PKB [9–14]. Global IP6K1 gene deletion in mice has revealed that IP_7 serves as a potent Akt/PKB inhibitor [9]. This has important consequences for insulin signaling in general and in particular the *in vivo* knockout of IP6K1 leads to enhanced Akt/PKB signaling in classic insulin-responsive tissues, including liver, skeletal muscle and white fat [8,9]. The resulting increased insulin sensitivity in these tissues creates an animal that overcomes high fat diet-induced insulin resistance [9].

We have previously studied IP_7 in the pancreatic β-cell where it acts both to prepare the β-cell for exocytosis [3,15], and to regulate initial insulin secretion [16]. In these cells glucose-stimulated IP_7 generation is largely mediated by IP6K1 that acts as a metabolic sensor transducing the glucose-mediated increase in the ATP/ADP ratio into first phase insulin secretion [16]. This is an important observation as it is the loss of first phase secretion that is characteristic of early diabetes and even pre-diabetes [17,18]. The importance of IP6K1 for β-cell exocytosis is in contrast to its negative impact on other insulin-sensitive tissues in

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which knockdown of IP6K1 leads to protection against insulin resistance due to enhanced Akt/PKB signaling.

The target(s) for IP_7 in β-cells are unknown, but based on the literature, Akt/PKB is a possible candidate. However, it is unlikely that inhibition of Akt/PKB by IP_7 can explain this inositol pyrophosphate's action in promoting insulin secretion as most reports suggest that inhibition of Akt/PKB has a negative effect on exocytosis [19–21], with one exception [22]. An important aspect of β-cell regulation and signal transduction is the fact that the secreted insulin feeds back on its own receptors thus re-initializing a second wave of signaling, including the activation of Akt/PKB [3,19,23]. This secondary signaling has been shown to be important for many aspects of β-cell function, including the biosynthesis and secretion of insulin itself [19,23]. Clearly, this unique feedback loop suggests that insulin resistance in the β-cell may also be a factor in the development of the diabetic phenotype.

We have now investigated the consequence of IP6K1 knockdown in pancreatic β-cells with respect to Akt/PKB signaling. The prediction, based on the results of IP6K1 knockdown in other insulin-sensitive cells [8,9], was that IP6K1 reduction and subsequent lowering of IP_7 would lead to enhanced Akt/PKB signaling. However, our experiments using glucose, the physiological stimulus of β-cells, revealed the complete opposite. IP_7 reduction via either RNAi mediated IP6K1 knockdown or the use of a specific pan-IP6K inhibitor, resulted in *decreased* rather than *increased* activation of Akt/PKB. The reason for this different outcome is the dominant positive action of IP_7 on Akt/PKB via the unique autocrine insulin feedback loop present in β-cells. This effect overcomes the direct inhibition of Akt/PKB by IP_7.

2. Experimental procedures

2.1. Chemicals

All cell culture reagents were obtained from Life Technologies (Stockholm, Sweden). Common chemicals and N2-(m-Trifluorobenzyl), N6-(p-nitrobenzyl) purine (TNP) were purchased either from Sigma (Stockholm, Sweden) or Merck KGaA (Darmstad, Germany), and insulin was obtained from Novo Nordisk (Denmark). Antibodies against phospho-Akt at T308 (#9275, #4056, #13038) and S473 (#9271), Akt (#9272) and β-actin (#8H10D10), were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). Antibodies against β-actin were from Thermofisher Scientific (MA5-15739) and Cell Signaling Technology (#8H10D10). The antibodies anti-phospho-IRS1 Y608 (#09-432) and anti-IRS1 (#06-248) were purchased from Millipore (Darmstadt, Germany) and the antibody anti-IP6K1 (#HPA040825) was from Sigma. Secondary antibodies were purchased from either Cell Signaling Technology, Thermofisher Scientific (MA, USA) or Sera care Life Sciences (Milford, MA, USA).

2.2. Cell culture

MIN6m9 cells, a generous gift from Dr. Seino [24], were cultured in complete DMEM containing 11 mM glucose, 10% FBS, 100 units/ml penicillin G, 100 μg/ml streptomycin-sulphate and 5 nl/ml β-mercaptoethanol in 5% CO_2 incubator at 37 °C.

2.3. RNA silencing

MIN6m9 cells were silenced as described previously [15]. siRNAs specific for IP6K1 (ID = 188560 and 71758) or non-targeting control (ID = 4611 and 4613) were purchased from Ambion Inc./ Thermofisher Scientific (Austin, TX).

2.4. Static incubation assays

MIN6m9 cells were seeded in complete DMEM. On the day of the experiment, the cells were preincubated for 1 h in modified KREBS buffer (119 mM NaCl, 4.6 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 0.15 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5 mM $NaHCO_3$, 0.5 mg/ml BSA, 20 mM HEPES pH 7.4) containing 0.5 mM glucose. For experiments involving TNP-treatment, 10 μM TNP or vehicle (DMSO) were added during the last 30 min of preincubation. After preincubation, the cells were stimulated with KREBS buffer containing either 0.5 mM glucose or 10 mM glucose for 3 min in the presence of vehicle (DMSO) or 10 μM TNP and in some cases exogenous insulin (58 ng/ml). The cells were then lysed for protein extraction followed by western blotting as described in the following section. In experiments where we investigated the effect of TNP on insulin secretion, the stimulation buffer was collected for insulin measurement and the cells were lysed with M-PER (Thermofisher Scientific) for protein normalization using a Pierce™ BCA protein assay kit (Thermofisher Scientific). Insulin was quantified using an AlphaLISA immunoassay kit (Perkin Elmer, Waltham, MA), according to the manufacturer's instructions [16].

2.5. Western blotting

Cells were lysed with RIPA buffer containing protease and phosphatase inhibitors (Roche Diagnostics, Stockholm, Sweden). The cell lysate was sonicated and centrifuged at 16,000*g* for 15 min. Protein content in the supernatant was measured by the BCA assay (Pierce™ BCA Protein Assay Kit). The same amount of proteins from each sample were denatured by heating in Laemmli sample buffer, separated by SDS–PAGE and transferred to nitrocellulose membranes [25]. Membranes were blocked with either 5% BSA or 5% skim milk in Tris-buffered saline containing 0.05% Tween 20 (TBST, pH 7.6) and then incubated with primary antibodies diluted in the same buffer at 4 °C overnight. The membranes were then washed with TBST for 1 h, incubated with HRP-conjugated secondary antibody [25], washed again with TBST and developed with SuperSignal West Femto Chemiluminescent Substrate (Thermofisher scientific). The chemiluminescence signal was detected by either a CCD camera or X-ray film exposure. The images were quantified by either ImageJ 1.48v or Image LabTM, BioRad. For quantifying different proteins from the same blot, the membrane was stripped and re-probed with respective antibodies. Western blotting results were normalized by sum of the replicates [26] for quantitative comparison and statistical analysis.

2.6. Statistical analysis

The data, expressed as means ± SEM, were statistically analyzed with GraphPad Prism software version 5.0. Detailed information on the statistical analysis of specific data sets is described in the respective figure legends and Supplemental Tables S1 and S2.

3. Results and discussion

3.1. IP6K1 serves to activate, not inhibit, Akt/PKB in insulin secreting cells

In β-cells the secretion of endogenous insulin is the physiologically relevant manner in which insulin stimulates insulin receptors and thus activates Akt/PKB. This is in contrast to how insulin is introduced in other cells. Insulin secretion in mouse β-cells including cell lines consists of two phases, with the early first phase dominating over the later second phase. Using insulin secreting MIN6m9 cells, we have previously established that glucose stimulation induces a peak in IP_7 production and insulin exocytosis after 3 min [16]. This increase in IP_7 levels and its subsequent stimulation of first phase insulin secretion are driven mainly by IP6K1 [16]. Existing studies, including those carried out with the IP6K1 knockout mice [8,9], have clearly shown an increased Akt/PKB activity when IP6K1 is knocked down in the classic insulin sensitive tissues including liver, skeletal muscle and white fat, as well as in a number of other cell types [8,9, 14, 27–33]. The effect of IP6K1/IP_7 knockdown on Akt/PKB activity is most marked in mice

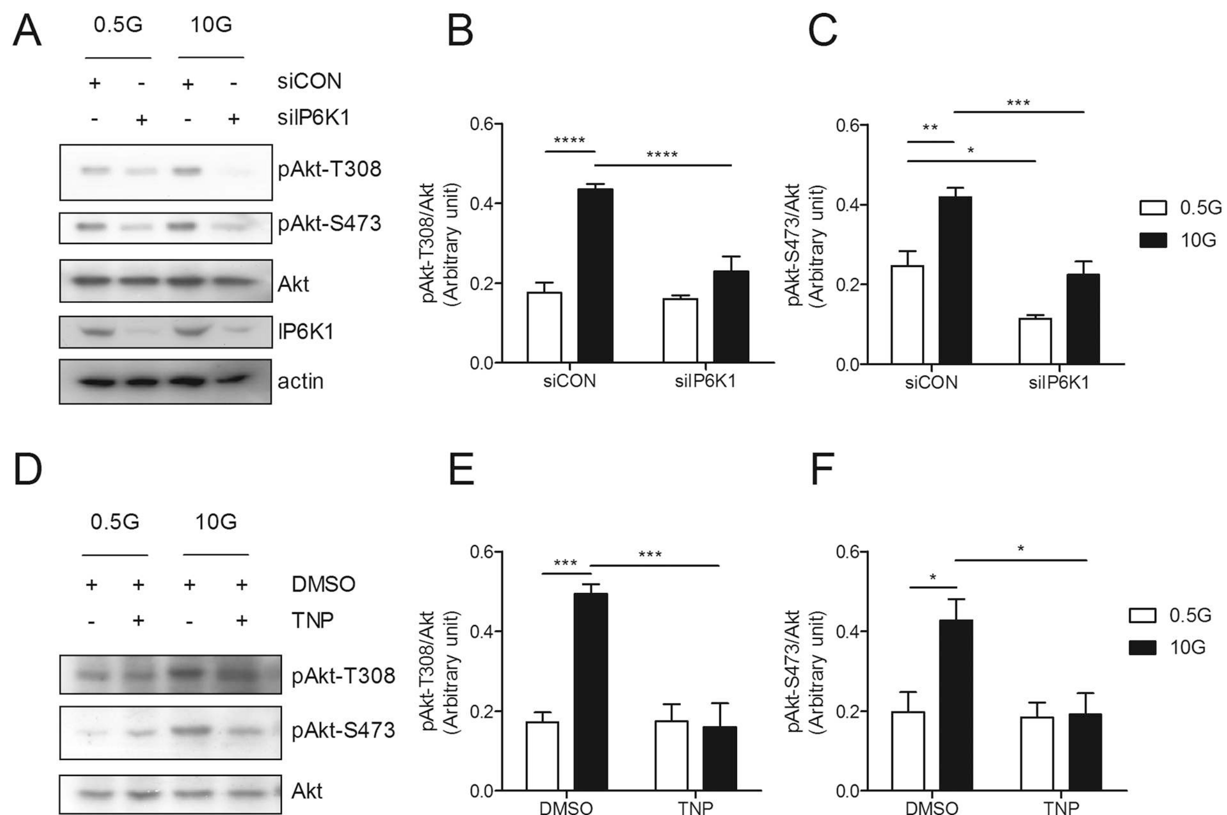

Fig. 1. Silencing of IP6K1 or treatment with TNP inhibits glucose-stimulated Akt/PKB phosphorylation in insulin secreting MIN6m9 cells. The involvement of IP_7 in Akt/PKB phosphorylation was studied in MIN6m9 cells using western blotting. Cells that were preincubated for 1 h at basal glucose condition (0.5 mM) were stimulated with 10 mM glucose for 3 min. For experiments involving TNP-treatment, 10 μM TNP or vehicle (DMSO) were also added during the last 30 min of preincubation. (A) Representative immunoblot out of six showing Akt phosphorylation obtained upon 0.5 mM glucose or 10 mM glucose treatment in control siRNA or IP6K1 siRNA treated cells. (B) Glucose mediated increase in Akt-T308 phosphorylation was abolished upon silencing IP6K1. (C) Silencing IP6K1 also decreased Akt-S473 phosphorylation under high glucose condition. (D) Representative immunoblot out of four showing Akt phosphorylation obtained upon 0.5 mM glucose or 10 mM glucose stimulation in DMSO or TNP treated cells. (E) TNP treatment decreased glucose mediated increase in Akt-T308 phosphorylation. (F) TNP treatment decreased Akt-S473 phosphorylation under high glucose condition. Data are presented as means ± SEM, $n = 6$ experiments (silencing) or $n = 4$ (TNP), $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$ using Two-way ANOVA (Supplemental Table S1). siCON, control siRNA; siIP6K1, IP6K1 siRNA; 0.5G, 0.5 mM glucose; 10G, 10 mM glucose.

under pathological conditions such as high-fat diet feeding [9,10], aging [14] or following various cardiac pathologies [30,31]. In some cell systems, IP6K1/IP_7 knockdown does not seem to impact Akt/PKB activity [34]. However, in this context insulin-sensitive β-cells were not investigated. Based on the above dynamics of glucose-induced IP_7 production we would anticipate a maximal impact of IP_7 on Akt/PKB at 3 min in MIN6m9 cells.

In order to examine the effect of IP6K1 knockdown on Akt/PKB we interrogated the two phosphorylation sites T308 and S473 using western blotting. These sites are established surrogates for the activation of Akt/PKB [35]. Glucose stimulation for 3 min caused a 2.5-fold increase in phosphorylation of T308 in MIN6m9 cells, indicating an increased Akt/PKB activity (Fig. 1A and B). There was also increased phosphorylation of the S473 site (Fig. 1A and C). Both Akt/PKB phosphorylation sites are activated by IP6K1 silencing in other insulin responsive cells (e.g. muscle) [8,9]. We discovered that silencing IP6K1 leads to a reduction, not activation, in Akt/PKB activity in β-cells (Fig. 1A–C), in contrast to other cell models. The magnitude of the inhibitory effect and the interplay with glucose stimulation was slightly different between the two phosphorylation sites. In the case of the T308 site (Fig. 1A and B), silencing IP6K1 prevented the increase in T308 phosphorylation after 3 min glucose stimulation. There was no effect on basal phosphorylation. In the case of the S473 site (Fig. 1A and C) glucose-stimulation also increased phosphorylation at this position, but to a lesser extent than at T308. It was also possible to observe a significant reduction in phosphorylation of this site upon silencing of IP6K1. IP6K1 silencing showed a similar tendency in Akt/PKB phosphorylation during a longer time of glucose stimulation (10 min), although Akt/PKB phosphorylation was less pronounced (Fig. S1). Overall, these data suggest that IP_7 may act by positively, rather than negatively, regulating Akt/PKB activity in β-cells.

3.2. Pharmacological IP6K inhibition by TNP also decreases Akt/PKB activation

RNAi mediated knockdown is a long-term approach to assess protein function and clearly the cell may adapt to this intervention. Therefore the disparity between our results and data from other insulin-responsive cells could be the result of specific longer-term and thus more indirect changes in β-cell physiology. To address this we used an alternative pharmacological approach. We subjected the cells to 3 min glucose stimulation in the presence or absence of the pan-IP6K inhibitor TNP. Fig. 1(D–F) illustrates that essentially the same pattern of reduced phosphorylation of T308 and S473 in Akt/PKB occurred in TNP treated MIN6m9 cells as in IP6K1 knockdown. Together these data substantiate the idea that the relationship between IP_7/IP6K1 and Akt/PKB phosphorylation is rather different in the pancreatic β-cell compared to several other cell types studied.

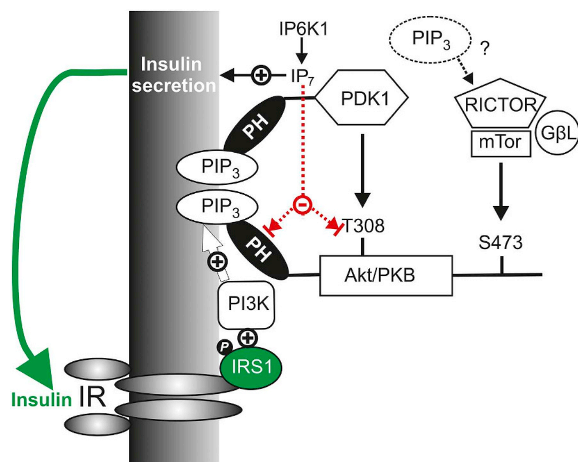

Fig. 2. Model of possible regulation of Akt/PKB by inositol pyrophosphates in β-cells. Proposed model illustrates that although IP_7 may inhibit Akt/PKB in pancreatic β-cells, its dominant effect could be to activate Akt/PKB, through the insulin feedback loop.

3.3. Is the impact of IP6K knockdown on Akt/PKB mediated via the insulin feedback loop?

At first sight our data suggest that IP_7 stimulates rather than inhibits Akt/PKB, diametrically opposing previous results in some other cells (e.g. white fat, skeletal muscle and liver). [8,9]. We decided to investigate this paradox further, focusing on one of the distinctive properties of the pancreatic β-cell, namely the insulin feedback loop. Our earlier work established that secreted insulin feeds back on its own receptor, thus re-initializing signaling events, particularly those instigated through PI3K and Akt/PKB [3,19,23,36]. Thus in the β-cell the compromised insulin secretion caused by silencing IP6K1 [16] should reduce the insulin signaling pathway that stimulates Akt/PKB (Model in Fig. 2). To clarify this we examined the phosphorylation of the insulin receptor substrate (IRS1) at the Y608 site, which is an initial activation step following the engagement of the insulin receptor by insulin. MIN6m9 cells exhibited a glucose-stimulated increase in insulin secretion (Fig. 3A) and IRS1 phosphorylation (Fig. 3B and C). Both these were reduced upon TNP treatment (Fig. 3), when production of IP_7 is curtailed [37]. This is unlikely to be due to interfering with a possible interaction of IP_7 with IRS1 phosphorylation because in the previous studies on muscle cells [9] reduction of IP_7 did not affect insulin-stimulated IRS1 phosphorylation. That is, there was no indication of a direct effect of IP_7 on IRS1. We propose that the dominant effect of IP_7 on the pancreatic β-cell Akt/PKB is to indirectly increase its kinase activity via increased exocytosis and insulin feedback loop (Fig. 2). Of course, this does not exclude the possibility that IP_7 may also directly inhibit Akt/PKB in these cells. In order to address this issue we needed to by-pass the impact of IP_7 on insulin secretion.

3.4. Direct effects of IP_7 on Akt-T308 phosphorylation

We examined independently whether in β-cells the mechanism of direct IP_7 action found in other insulin-sensitive cells is masked by the autocrine insulin feedback. We studied the effects of the TNP treatment in cells exposed to exogenous insulin (58 ng/ml) under basal conditions (0.5 mM glucose) in order to mimic the extracellular insulin levels reached during glucose stimulation. Fig. 4A shows that exogenous insulin caused a significant elevation in T308 phosphorylation in control cells. This increase in phosphorylation was similar to that elicited by glucose in the same experiment. However, when cells were stimulated with insulin in the presence of TNP this elevation in T308 phosphorylation was significantly enhanced, whereas there was no difference in S473 phosphorylation (Fig. 4B). These findings are consistent with the observations performed in other cell systems, e.g. Fig. S2A in [9]. Our data thus indicate that IP_7 inhibits T308 phosphorylation also in β-cells. They confirm the idea that the relationship between IP6K1 and Akt/PKB is not fundamentally different from cells examined in other studies, but that the autocrine insulin feedback loop driven by IP_7-mediated exocytosis is the dominant factor in determining the overall impact of IP_7 on Akt/PKB activity in β-cells.

4. Conclusions

Our findings expose a unique interplay between IP_7 and Akt/PKB in pancreatic β-cells. Two elements create this distinctive situation. The first is the importance of IP_7 in insulin secretion [3,16]. The second is the autocrine feedback of the secreted insulin on β-cell insulin receptors, which activates Akt/PKB [23]. When you combine these two elements, reducing IP_7 concentrations also reduces, indirectly, Akt/PKB signaling. This feedback effect dominates over the inhibitory action the pyrophosphate has on Akt/PKB (see model in Fig. 2). Therefore, our data are not contradictory to the established thinking regarding IP_7 action on Akt/PKB, but reflect inositol pyrophosphate metabolism in a more complex biological setting. These results also imply that

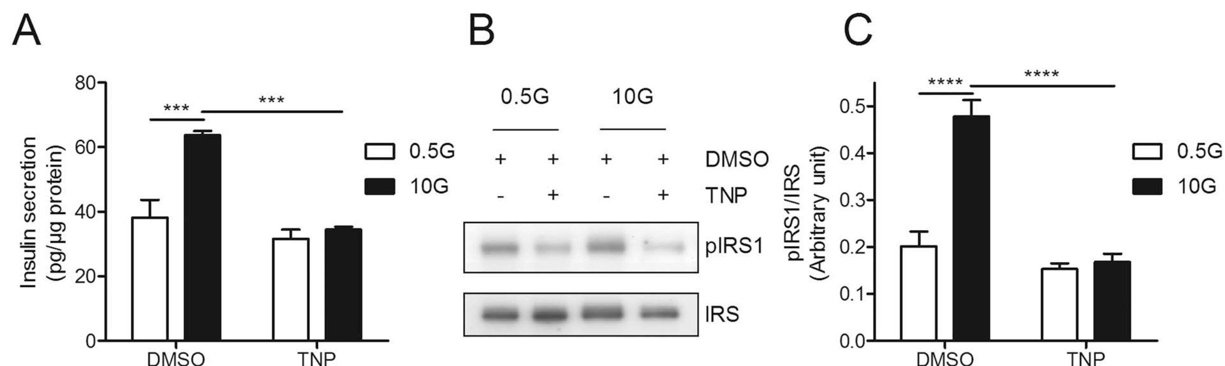

Fig. 3. The pan-IP6K inhibitor TNP blocks glucose-stimulated insulin secretion and insulin-mediated activation of IRS1 in MIN6m9 cells. (A) Stimulation of MIN6m9 cells with 10 mM glucose induced a significant increase in insulin secretion. In contrast, in the presence of 10 µM TNP, 10 mM glucose did not induce any secretion. Data are presented as means ± SEM, n = 4 experiments, ***$p < .001$ using Two-way ANOVA (Supplemental Table S1). The TNP effect on insulin-mediated IRS1 phosphorylation was studied in MIN6m9 cells by immunoblotting. (B) Representative immunoblot out of four showing IRS1 phosphorylation obtained upon 0.5 mM glucose or 10 mM glucose treatment in DMSO or TNP treated cells. (C) There was a significant increase in IRS1 phosphorylation upon 10 mM glucose stimulation for 3 min in the samples treated with vehicle (DMSO). Inhibiting the glucose-mediated increase in insulin secretion by 10 µM TNP treatment (A), reduced IRS1 phosphorylation. Data are presented as means ± SEM, n = 4 experiments, ***$p < .001$ using Two-way ANOVA (Supplemental Table S1). 0.5G, 0.5 mM glucose; 10G, 10 mM glucose.

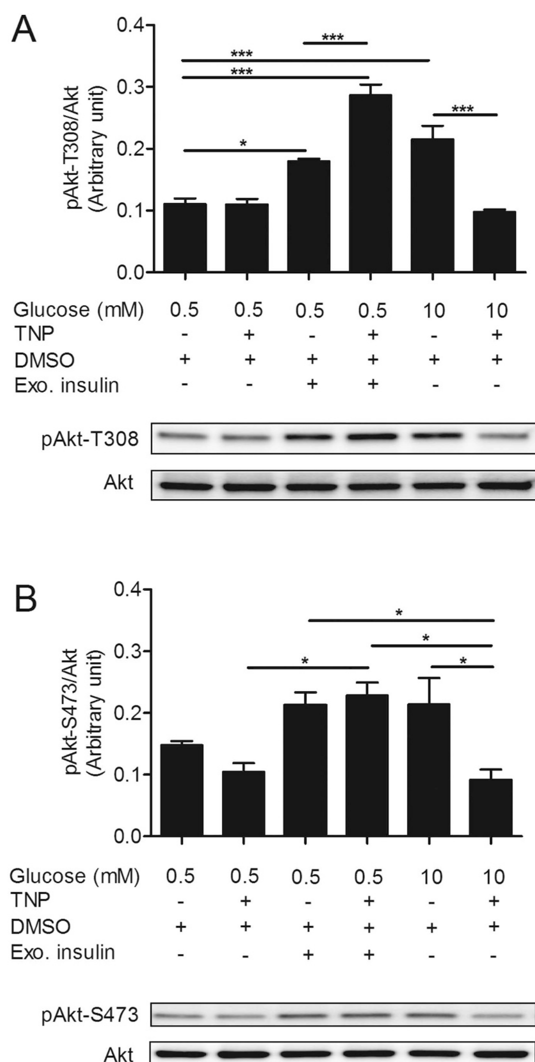

Fig. 4. Treatment with TNP enhances the effects of exogenous insulin on Akt phosphorylation at basal glucose. Akt-activation was studied after stimulation with exogenous insulin (Exo. insulin) at low glucose (0.5 mM) for 3 min. (A) Stimulation with exogenous insulin (58 ng/ml) increased Akt-T308 phosphorylation in vehicle (DMSO)-treated MIN6m9 cells compared to controls, mimicking the activation induced by stimulation with 10 mM glucose. The effect of exogenous insulin was enhanced by treatment with the pan-IP6K inhibitor TNP (10 μM). In contrast, TNP abolished Akt-activation induced by 10 mM glucose stimulation, in agreement with its inhibitory effect on the release of endogenous insulin by the β-cells (see Fig. 3A). (B) Analysis of Akt-S473 phosphorylation of the sample set in panel A. Data are presented as means ± SEM, $n = 3$ experiments, $*p < .05$, $***p < .001$ using One-way ANOVA (see Supplemental Table S2 for the comprehensive statistical analysis).

inhibiting the production of IP_7 in pancreatic β-cells could lead to an insulin resistance phenotype in these cells. However, one must be careful to extrapolate these *in vitro* findings to the situation *in vivo* which will be necessarily more intricate, both in terms of normal physiology and metabolic diseases [8,11].

Considering treatment strategies for obesity and type 2 diabetes, IP6K inhibition seems to be beneficial because it decreases insulin resistance in peripheral tissues, as indicated by the ability of TNP to ameliorate diet induced obesity in mice [10]. In terms of the β-cell, our concern is that IP6K1 inhibition disrupts insulin secretion. This seems to be compensated by increase of insulin sensitivity in peripheral tissues [9]. It is theoretically possible that TNP treatment may also prevent hypersecretion of insulin, a putative contributor to the insulin resistance. However, in the later stages of the disease, when β-cell insulin secretion is significantly decreased, further reduction of secretion mediated by TNP, or future IP6K inhibiting drugs, would be undesirable. In conclusion, potential modulation of IP6K activity in diabetic patients will have to be matched carefully with the stage of disease development.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2019.02.003.

References

[1] C. Azevedo, A. Saiardi, Eukaryotic phosphate homeostasis: the inositol pyrophosphate perspective, Trends Biochem. Sci. 42 (3) (2017) 219–231.
[2] C. Azevedo, Z. Szijgyarto, A. Saiardi, The signaling role of inositol hexakisphosphate kinases (IP6Ks), Adv. Enzym. Regul. 51 (1) (2011) 74–82.
[3] C.J. Barker, P.O. Berggren, New horizons in cellular regulation by inositol polyphosphates: insights from the pancreatic beta-cell, Pharmacol. Rev. 65 (2) (2013) 641–669.
[4] A. Chakraborty, S. Kim, S.H. Snyder, Inositol pyrophosphates as mammalian cell signals, Sci. Signal. 4 (188) (2011) re1.
[5] A. Saiardi, Cell signalling by inositol pyrophosphates, Subcell. Biochem. 59 (2012) 413–443.
[6] S.B. Shears, Diphosphoinositol polyphosphates: metabolic messengers? Mol. Pharmacol. 76 (2) (2009) 236–252.
[7] T. Wundenberg, G.W. Mayr, Synthesis and biological actions of diphosphoinositol phosphates (inositol pyrophosphates), regulators of cell homeostasis, Biol. Chem. 393 (9) (2012) 979–998.
[8] A. Chakraborty, The inositol pyrophosphate pathway in health and diseases, Biol. Rev. Camb. Philos. Soc. 93 (2018) 1203–1227.
[9] A. Chakraborty, M.A. Koldobskiy, N.T. Bello, M. Maxwell, J.J. Potter, K.R. Juluri, D. Maag, S. Kim, A.S. Huang, M.J. Dailey, M. Saleh, A.M. Snowman, T.H. Moran, E. Mezey, S.H. Snyder, Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain, Cell 143 (6) (2010) 897–910.
[10] S. Ghoshal, Q. Zhu, A. Asteian, H. Lin, H. Xu, G. Ernst, J.C. Barrow, B. Xu, M.D. Cameron, T.M. Kamenecka, A. Chakraborty, TNP [N2-(m-Trifluorobenzyl), N6-(p-nitrobenzyl)purine] ameliorates diet induced obesity and insulin resistance via inhibition of the IP6K1 pathway, Mol. Metab. 5 (10) (2016) 903–917.
[11] R.W. Mackenzie, B.T. Elliott, Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 diabetes, Diabetes Metab. Syndr. Obes. 7 (2014) 55–64.
[12] I. Pavlovic, D.T. Thakor, J.R. Vargas, C.J. McKinlay, S. Hauke, P. Anstaett, R.C. Camuna, L. Bigler, G. Gasser, C. Schultz, P.A. Wender, H.J. Jessen, Cellular delivery and photochemical release of a caged inositol-pyrophosphate induces PH-domain translocation in cellulo, Nat. Commun. 7 (2016) 10622.
[13] A. Prasad, Y. Jia, A. Chakraborty, Y. Li, S.K. Jain, J. Zhong, S.G. Roy, F. Loison, S. Mondal, J. Sakai, C. Blanchard, S.H. Snyder, H.R. Luo, Inositol hexakisphosphate kinase 1 regulates neutrophil function in innate immunity by inhibiting phosphatidylinositol-(3,4,5)-trisphosphate signaling, Nat. Immunol. 12 (8) (2011) 752–760.
[14] Z. Zhang, C. Zhao, B. Liu, D. Liang, X. Qin, X. Li, R. Zhang, C. Li, H. Wang, D. Sun, F. Cao, Inositol pyrophosphates mediate the effects of aging on bone marrow mesenchymal stem cells by inhibiting Akt signaling, Stem Cell Res Ther 5 (2) (2014) 33.
[15] C. Illies, J. Gromada, R. Fiume, B. Leibiger, J. Yu, K. Juhl, S.N. Yang, D.K. Barma, J.R. Falck, A. Saiardi, C.J. Barker, P.O. Berggren, Requirement of inositol pyrophosphates for full exocytotic capacity in pancreatic beta cells, Science 318 (5854) (2007) 1299–1302.
[16] S.S. Rajasekaran, J. Kim, G.C. Gaboardi, J. Gromada, S.B. Shears, K.T. Dos Santos, E.L. Nolasco, S.S. Ferreira, C. Illies, M. Köhler, C. Gu, S.H. Ryu, J.O. Martins, E. Darè, C.J. Barker, P.O. Berggren, Inositol hexakisphosphate kinase 1 is a

metabolic sensor in pancreatic beta-cells, Cell. Signal. 46 (2018) 120–128.
[17] E. Cerasi, R. Luft, The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus, Acta Endocrinol. 55 (2) (1967) 278–304.
[18] B. Ahren, Type 2 diabetes, insulin secretion and beta-cell mass, Curr. Mol. Med. 5 (3) (2005) 275–286.
[19] B. Leibiger, T. Moede, S. Uhles, C.J. Barker, M. Creveaux, J. Domin, P.O. Berggren, I.B. Leibiger, Insulin-feedback via PI3K-C2alpha activated PKBalpha/Akt1 is required for glucose-stimulated insulin secretion, FASEB J. 24 (6) (2010) 1824–1837.
[20] X. Cui, G. Yang, M. Pan, X.N. Zhang, S.N. Yang, Akt signals upstream of L-type calcium channels to optimize insulin secretion, Pancreas 41 (1) (2012) 15–21.
[21] B.O. Le, G. Queniat, V. Gmyr, J. Kerr-Conte, B. Lefebvre, F. Pattou, mTORC1 and mTORC2 regulate insulin secretion through Akt in INS-1 cells, J. Endocrinol. 216 (1) (2013) 21–29.
[22] K. Aoyagi, M. Ohara-Imaizumi, C. Nishiwaki, Y. Nakamichi, K. Ueki, T. Kadowaki, S. Nagamatsu, Acute inhibition of PI3K-PDK1-Akt pathway potentiates insulin secretion through upregulation of newcomer granule fusions in pancreatic beta-cells, PLoS One 7 (10) (2012) e47381.
[23] I.B. Leibiger, B. Leibiger, P.O. Berggren, Insulin signaling in the pancreatic beta-cell, Annu. Rev. Nutr. 28 (2008) 233–251.
[24] K. Minami, H. Yano, T. Miki, K. Nagashima, C.Z. Wang, H. Tanaka, J.I. Miyazaki, S. Seino, Insulin secretion and differential gene expression in glucose-responsive and -unresponsive MIN6 sublines, Am. J. Physiol. Endocrinol. Metab. 279 (4) (2000) E773–E781.
[25] J. Kim, Y.S. Choi, S. Lim, K. Yea, J.H. Yoon, D.J. Jun, S.H. Ha, J.W. Kim, J.H. Kim, P.G. Suh, S.H. Ryu, T.G. Lee, Comparative analysis of the secretory proteome of human adipose stromal vascular fraction cells during adipogenesis, Proteomics 10 (3) (2010) 394–405.
[26] A. Degasperi, M.R. Birtwistle, N. Volinsky, J. Rauch, W. Kolch, B.N. Kholodenko, Evaluating strategies to normalise biological replicates of Western blot data, PLoS One 9 (1) (2014) e87293.
[27] A. Chakraborty, C. Latapy, J. Xu, S.H. Snyder, J.M. Beaulieu, Inositol hexakisphosphate kinase-1 regulates behavioral responses via GSK3 signaling pathways, Mol. Psychiatry 19 (3) (2014) 284–293.
[28] Q. Zhu, S. Ghoshal, A. Rodrigues, S. Gao, A. Asterian, T.M. Kamenecka, J.C. Barrow, A. Chakraborty, Adipocyte-specific deletion of Ip6k1 reduces diet-induced obesity by enhancing AMPK-mediated thermogenesis, J. Clin. Invest 126 (11) (2016) 4273–4288.
[29] Q. Zhu, S. Ghoshal, R. Tyagi, A. Chakraborty, Global IP6K1 deletion enhances temperature modulated energy expenditure which reduces carbohydrate and fat induced weight gain, Mol. Metab. 6 (1) (2017) 73–85.
[30] D. Sun, S. Li, H. Wu, M. Zhang, X. Zhang, L. Wei, X. Qin, E. Gao, Oncostatin M (OSM) protects against cardiac ischaemia/reperfusion injury in diabetic mice by regulating apoptosis, mitochondrial biogenesis and insulin sensitivity, J. Cell Mol. Med. 19 (6) (2015) 1296–1307.
[31] Z. Zhang, D. Liang, X. Gao, C. Zhao, X. Qin, Y. Xu, T. Su, D. Sun, W. Li, H. Wang, B. Liu, F. Cao, Selective inhibition of inositol hexakisphosphate kinases (IP6Ks) enhances mesenchymal stem cell engraftment and improves therapeutic efficacy for myocardial infarction, Basic Res. Cardiol. 109 (4) (2014) 417.
[32] M. Wu, L.S. Chong, S. Capolicchio, H.J. Jessen, A.C. Resnick, D. Fiedler, Elucidating diphosphoinositol polyphosphate function with nonhydrolyzable analogues, Angew Chem. Int. Ed. Engl. 53 (28) (2014) 7192–7197.
[33] M. Wu, B.E. Dul, A.J. Trevisan, D. Fiedler, Synthesis and characterization of non-hydrolysable diphosphoinositol polyphosphate second messengers, Chem. Sci. 4 (1) (2013) 405–410.
[34] C. Gu, M.A. Stashko, A.C. Puhl-Rubio, M. Chakraborty, A. Chakraborty, S.V. Frye, K.H. Pearce, X. Wang, S.B. Shears, H. Wang, Inhibition of inositol polyphosphate kinases by quercetin and related flavonoids: a structure-activity analysis, J. Med. Chem. (2019).
[35] J.R. Bayascas, D.R. Alessi, Regulation of Akt/PKB Ser473 phosphorylation, Mol. Cell 18 (2) (2005) 143–145.
[36] J. Yu, P.O. Berggren, C.J. Barker, An autocrine insulin feedback loop maintains pancreatic beta-cell 3-phosphorylated inositol lipids, Mol. Endocrinol. 21 (11) (2007) 2775–2784.
[37] U. Padmanabhan, D.E. Dollins, P.C. Fridy, J.D. York, C.P. Downes, Characterization of a selective inhibitor of inositol hexakisphosphate kinases: use in defining biological roles and metabolic relationships of inositol pyrophosphates, J. Biol. Chem. 284 (16) (2009) 10571–10582.