

Original Paper

Activation of Extracellular Signal-Regulated Protein Kinases 1 and 2 (ERK1/2) by Free Fatty Acid Receptor 1 (FFAR1/GPR40) Protects from Palmitate-Induced Beta Cell Death, but Plays no Role in Insulin Secretion

Madhura Panse^a Felicia Gerst^{a,b} Gabriele Kaiser^{a,b} Charlott-Amélie Teutsch^a
Rebecca Dölker^a Robert Wagner^{a,b} Hans-Ulrich Häring^{a,b} Susanne Ullrich^{a,b}

^aUniversity of Tübingen, Department of Internal Medicine, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, Tübingen, Germany

^bInstitute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen (IDM) and German Center for Diabetes Research (DZD e.V.) Tübingen, Germany

Key Words

Fatty acid receptor-1 • GPR40/FFAR1 • Insulin secretion • Beta-cell apoptosis • ERK1/2 • Lipotoxicity

Abstract

Aims: GPR40/FFAR1 mediates palmitate-induced stimulation of insulin secretion but its involvement in lipotoxicity is controversial. Our previous observations suggest that FFAR1/GPR40 agonists protect against lipotoxicity although the underlying mechanism remains elusive. The present study examines the role of ERK1/2 and GPR40/FFAR1 in palmitate-induced stimulation of insulin secretion and beta cell death. **Methods:** Insulin secretion of INS-1E cells was measured by radioimmunoassay. Protein phosphorylation was examined on Western blots. Apoptosis was assessed by TUNEL staining. **Results:** Palmitate and the GPR40/FFAR1 agonist TUG-469 increased phosphorylation of ERK1/2 at low (2.8 mmol/L) and high (12 mmol/L) glucose but stimulated insulin secretion only at high glucose. The MEK1 inhibitor PD98059 significantly reduced phosphorylation of ERK1/2 but did not reverse the stimulation of secretion induced by glucose, palmitate or TUG-469. PD98059 rather augmented glucose-induced secretion. Prolonged exposure to palmitate stimulated apoptosis, an effect counteracted by TUG-469. PD98059 accentuated palmitate-induced apoptosis and reversed TUG-469-mediated inhibition of cell death. **Conclusions:** Activation of ERK1/2 by palmitate and GPR40/FFAR1 agonist correlates neither with stimulation of insulin secretion nor with induction of apoptosis. The results suggest a significant anti-apoptotic role of ERK1/2 under conditions of lipotoxicity.

Copyright © 2015 S. Karger AG, Basel

Susanne Ullrich

University of Tübingen, IDM and Department of Internal Medicine IV, Division of Endocrinology, Diabetology, Vascular Medicine, Nephrology and Clinical Chemistry, Otfried-Müller-Strasse 10, D-72076 Tübingen (Germany)
Tel. +49-7071-29 84471, E-Mail susanne.ullrich@med.uni-tuebingen.de

Introduction

The importance of FFAR1/GPR40 in fatty acid-mediated stimulation of insulin secretion is widely accepted [1, 2]. The role in lipotoxicity, however, is controversially discussed. Our previous observations indicate a protective effect of FFAR1/GPR40 against lipotoxicity [3]. However, other studies suggested that inhibition of FFAR1/GPR40 counteracted fatty acid-induced beta cell death [4, 5]. Using recombinantly expressed receptors, it has been proposed that GPR40/FFAR1 activates the extracellular signal-regulated protein kinases 1 and 2, ERK1/2 [6].

The role of MAPK signalling pathways is manifold [7]. Activation of ERK1/2 by growth factors, including IGF-1, stimulates early genes and promotes proliferation. ERK1/2 further exerts effects on cell survival and differentiation. In insulin secreting cells stimulation of ERK1/2 parallels insulin secretion induced by glucose, by substances which increase cAMP (GLP-1, GIP) and by phorbol esters which activate PKCs, suggesting an involvement of ERK1/2 [8, 9]. IGF-1, glucose and incretins have additive effects on ERK1/2 activation [10]. Stimulation of ERK1/2 by IGF-1 is mediated via ras, raf-1 and MEK1 and is independent of an increase in cytosolic Ca^{2+} [11]. On the contrary, activation of ERK1/2 by glucose depends on the increase in cytosolic Ca^{2+} and on cAMP-induced stimulation of PKA which directly phosphorylates raf-1 [10, 12].

The function of ERK1/2 is not completely understood due to contradictory observations. The MEK1 inhibitor PD98059 did not affect glucose-induced insulin secretion in INS-1E cells [13]. In contrast, inhibition of glucose-dependent ERK1/2 phosphorylation upon chronic exposure to palmitate was linked to a concomitant reduction in glucose-induced insulin secretion in Min6 cells [14].

Furthermore, numerous observations suggested an anti-apoptotic role of ERK1/2, both in Min6 and INS-1E cells [15-19]. In line, ERK1/2 was shown to counteract CHOP expression, an ER-stress marker which strongly correlates with beta cell death [11, 20, 21]. However, these findings were challenged in isolated human islets, where inhibition of ERK1/2 by PD98059 antagonized glucotoxicity [22]. Thus, the involvement of ERK1/2 in insulin secretion and beta cell apoptosis remains elusive.

This study aims to understand the role of ERK1/2 in the regulation of insulin secretion and induction of apoptosis during acute and chronic exposure of insulin secreting INS-1E cells to the saturated fatty acid palmitate and the GPR40/FFAR1 agonist TUG-469.

Materials and Methods

Materials

INS-1E cells were kindly provided by C.B. Wollheim and P. Maechler (University of Geneva, Geneva, Switzerland) and TUG-469 was a kind gift from T. Ulven (Southern University of Denmark, Odense, Denmark). Fetal calf serum (FCS) was purchased from Serva (Heidelberg, Germany); MEK1 inhibitor PD98059 and antibodies against PKB (#9272), P-Thr183/Tyr185-JNK (#9251), P-Ser73-cJun (#9164), ERK1/2 (#9102), P-Thr202/Tyr204-ERK1/2 (#9101) and tubulin (#2148) were from Cell Signalling Technology Europe (Leiden, The Netherlands). The antibody against GAPDH (V-18, #sc-20357) was from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany), horseradish peroxidase-coupled anti-rabbit IgG was from GE Healthcare (Buckinghamshire, U.K.), TUNEL assay from Roche Diagnostics GmbH (Basel, Switzerland). All other chemicals were purchased from Sigma-Aldrich (Deisenhofen, Germany).

Methods

Cell culture. INS-1E cells were kept under standard culture condition in RPMI1640 medium containing 11 mmol/L glucose and supplemented with 10% FCS, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 1 mmol/L Na-pyruvate and 10 μ mol/L 2-mercaptoethanol. Cells were treated with the MEK1 inhibitor PD98059 (10 μ mol/L) 1h prior to and during exposure to palmitate, TUG-469 or phorbol myristate acetate (PMA).

Insulin secretion. Insulin secretion was measured in static incubation by using modified Krebs-Ringer buffer (KRB), containing in mmol/L: 135 NaCl, 4.8 KCl, 1.2 MgSO₄, 1.3 CaCl₂, 1.2 KH₂PO₄, 5 NaHCO₃, 2.8

glucose, 10 HEPES, and 5 g/L bovine serum albumin (fatty acid free; Sigma-Aldrich) pH=7.4. The cells were preincubated for 1h at 2.8 mmol/L glucose and incubated for another hour in the presence of test substances. Secreted insulin and insulin content of the cells after extraction in acid ethanol (0.18 mmol/L HCl in 70% ethanol) were measured by radioimmunoassay (Millipore, Billerica, MA, USA). A stock solution of palmitate in DMSO (100 mmol/L) was diluted in FCS (containing 5 % albumin) or coupled to BSA (5% in KRB) such that the concentration of palmitate was 6 mmol/L. These solutions were diluted as required for the respective experiments keeping the palmitate/albumin ratio constant. The agonist TUG-469 was used at low albumin (FCS) concentration (0.05 % (w/v)) since higher concentrations of albumin inhibit TUG-469 effects on secretion (unpublished observations).

TUNEL assay. Cells were seeded onto glass cover slips and cultured in the presence of test substances as indicated. After fixation with 4% PFA for 1h cells were permeabilized with 0.1% Triton X-100 in PBS and fractionated DNA was detected using a commercial kit (Roche Diagnostics). Nuclei were stained with 1 μ mol/L TO-PRO3 or 0.1 μ g/mL DAPI in PBS.

Western blotting. After treatment with test substances as indicated, cells were lysed in a buffer containing 125 mmol/L NaCl, 1% (v/v) Triton X-100, 0.5% Na-deoxycholate, 0.1% SDS, 10 mmol/L EDTA, 25 mmol/L HEPES, pH 7.3, 10 mmol/L NaPP, 10 mmol/L NaF, 1 mmol/L Na-vanadate, 10 μ g/mL pepstatin A, 10 μ g/mL aprotinin and 0.1 mmol/L PMSF as described previously [23]. Proteins (30 μ g) of a 10.000g supernatant were subjected to SDS-PAGE and blotted on nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). Membranes were incubated overnight with primary antibodies (1:1000) followed by incubation with the secondary anti-rabbit IgG (1:2000). Results were quantified using ImageJ software (NIH, USA).

Statistical analysis. Data are presented as means \pm SEM. Significance was tested using Student's t-test or ANOVA followed by Dunnett's or Newmann-Keul's post-tests as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

Activation of ERK1/2 is not critical for palmitate- and TUG-469-induced insulin secretion

In order to correlate stimulation of ERK1/2 to insulin secretion, the effects of palmitate on ERK1/2 phosphorylation and insulin secretion were assessed in the absence and presence of the MEK1 inhibitor PD98059, 10 μ mol/L (Fig. 1A-C). Palmitate stimulated phosphorylation of ERK1/2 at 2.8 mmol/L glucose without inducing secretion. High glucose, 12 mmol/L, increased ERK1/2 phosphorylation along with an increase in secretion. Palmitate further augmented the glucose effect on secretion and phosphorylation of ERK1/2 remained high. However, PD98059 did not inhibit glucose- and palmitate-stimulation of secretion despite a significant reduction of ERK1/2 phosphorylation. Interestingly, glucose-induced insulin secretion was significantly augmented by PD98059. Furthermore the effect of palmitate on ERK1/2 phosphorylation and insulin secretion was mimicked by the selective FFAR1/GPR40 agonist TUG-469, 10 μ mol/L (Fig. 2A-C). Again, the inhibition by PD98059 did not lower insulin secretion. Of note, this effect of PD98059 was specific as the inhibitor did not significantly affect phosphorylation of JNK and its substrate, c-Jun (Fig. 3A-D).

These results demonstrate that stimulation of ERK1/2 does not correlate with stimulation of insulin secretion suggesting, therefore, that ERK1/2 does not play a major role in palmitate-mediated, GPR40/FFAR1-dependent stimulation of insulin secretion in INS-1E cells.

Activation of ERK1/2 is not sufficient to induce beta cell death

To test whether ERK1/2 is activated under lipotoxic conditions, INS-1E cells were exposed to 600 μ mol/L palmitate dissolved in culture medium containing 12 mmol/L glucose and supplemented with 10% FCS (Fig. 4A,B). In parallel cells were treated with the phorbol ester, PMA (100 nmol/L). Both, palmitate and PMA significantly increased phosphorylation of ERK1/2 (Fig. 4A,B). The effects, however, were transient and ERK1/2 phosphorylation returned to basal levels after 24h exposure to PMA as well as palmitate. Palmitate but not

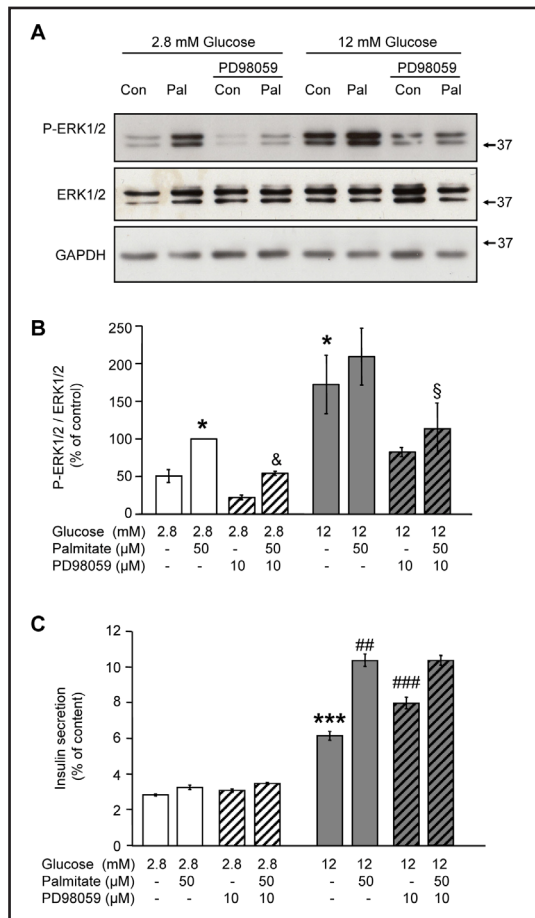


Fig. 1. MEK1 inhibition antagonizes activation of ERK1/2 without reducing stimulation of insulin secretion. INS-1E cells were incubated in KRB supplemented with 0.05% BSA, glucose and test substances as indicated: 2.8 (white bars) and 12 mmol/L (grey bars) glucose, palmitate (Pal, 50 μ mol/L), MEK1 inhibitor PD98059 (10 μ mol/L, hatched bars). (A) Representative Western blots of P-ERK1/2 and ERK1/2; GAPDH used as house keeping gene. (B) Relative changes of ERK1/2 phosphorylation expressed as means \pm SEM of n=3 independent experiments. (C) INS-1E cells were incubated in parallel for insulin secretion measurements as described in detail in Material and Methods. Results are presented as means \pm SEM of n=3 independent experiments. *p<0.05, ***p<0.001 denotes significance to 2.8 mmol/L glucose; #p<0.05, ##p<0.005, ###p<0.001 denotes significance to 12 mmol/L glucose; §p<0.05 denotes significance to 12 mmol/L glucose+50 μ mol/L palmitate; &p<0.05 denotes significance to 2.8 mmol/L glucose+50 μ mol/L palmitate.

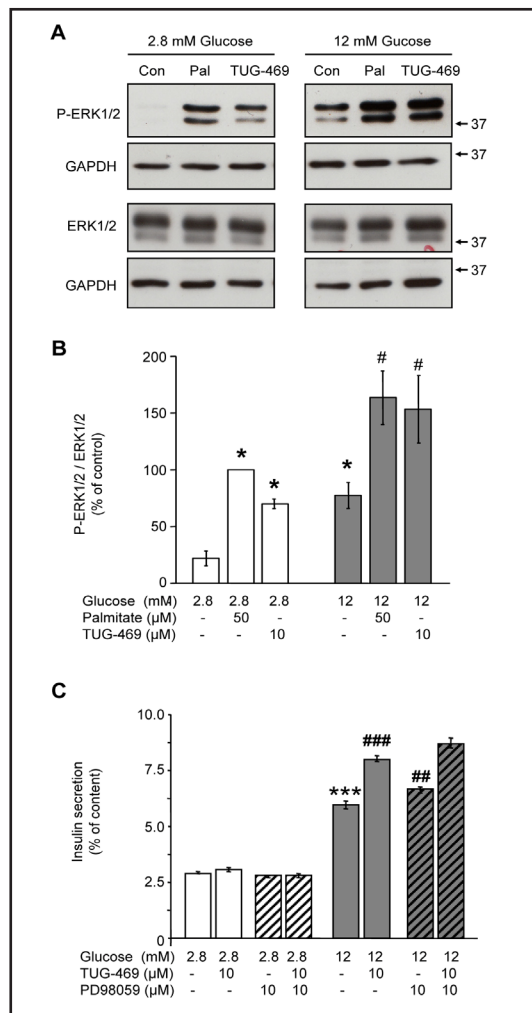


Fig. 2. ERK1/2 is activated by TUG-469 but plays no role in stimulation of insulin secretion. INS-1E cells were exposed for 1h to test substances in KRB supplemented with 0.05% BSA and 2.8 mmol/L glucose (white bars) or 12 mmol/L glucose (grey bars) as indicated: palmitate (Pal, 50 μ mol/L) and TUG-469 (10 μ mol/L). (A) Representative Western blots for P-ERK1/2 and ERK1/2; GAPDH used as house keeping gene. (B) Relative changes of ERK1/2 phosphorylation expressed as means \pm SEM of n=4 independent experiments. (C) INS-1E cells were incubated in parallel for insulin secretion. Results are presented as means \pm SEM of n=3 independent experiments. *p<0.05, ***p<0.001 denote significance to 2.8 mmol/L glucose; # p<0.05, ##p<0.005, ###p<0.001 indicates significance to 12 mmol/L glucose.

PMA increased the percentage of TUNEL stained cells, suggesting that ERK1/2 activation is not responsible for the induction of apoptosis. This conclusion is further corroborated by the

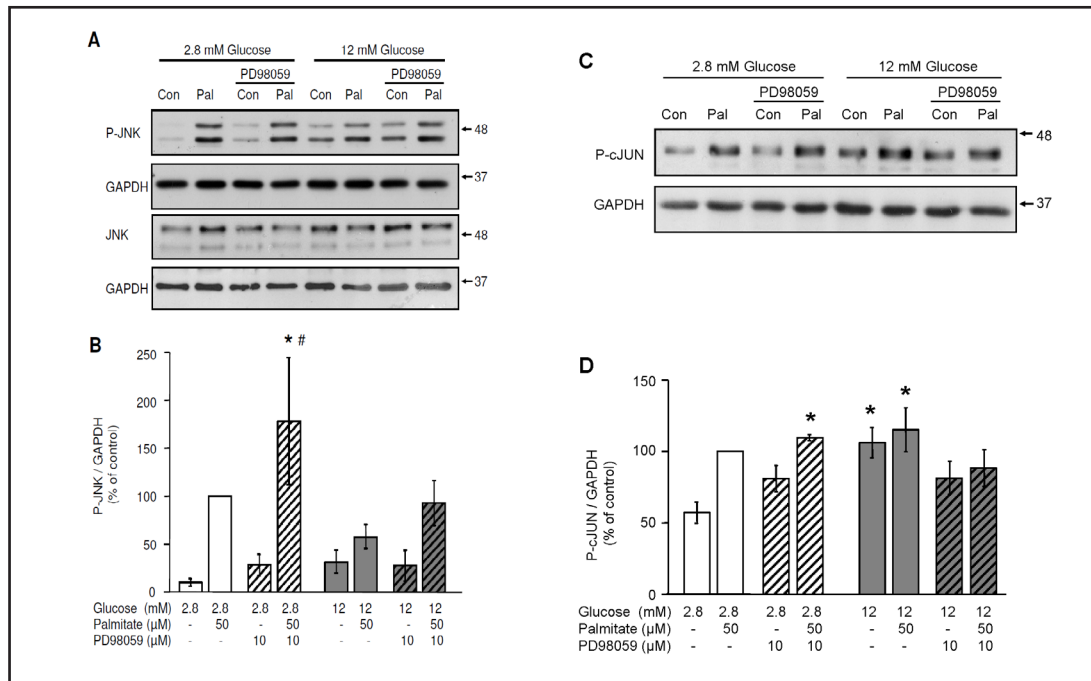
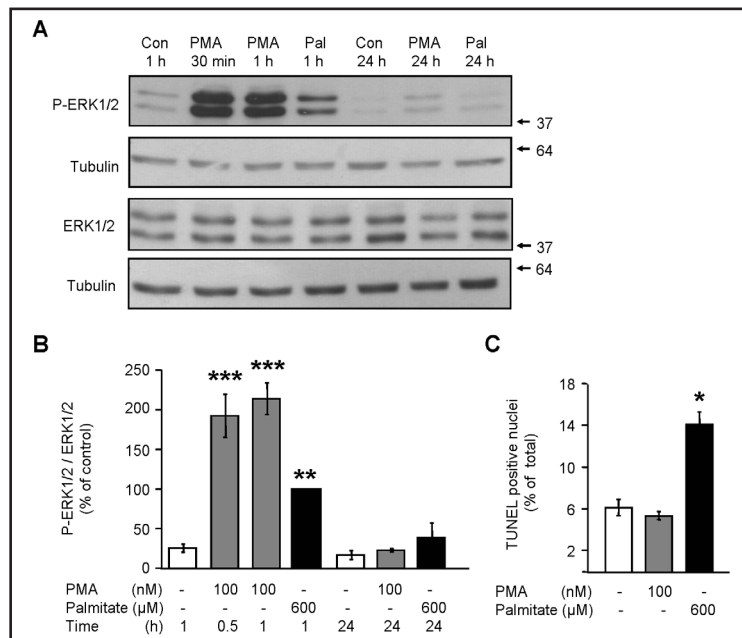


Fig. 3. MEK1 inhibition does not affect activation of JNK and its downstream target c-Jun. Representative Western blot analyses of (A) P-JNK and JNK and (C) P-c-Jun of INS-1E cell homogenates from the same experiments as shown in Fig. 2 and (B and D) respective means \pm SEM of n=3-4 independent experiments. *p<0.05 denotes significance against 2.8 mmol/L glucose, #p<0.05 against 2.8 mmol/L glucose + PD98059.

Fig. 4. ERK1/2 stimulation by palmitate but not by the phorbol ester PMA is accompanied by induction of cell death. INS-1E cells were treated in cell culture with PMA, 100 nmol/L (grey bars), and palmitate, 600 μ mol/L (Pal, black bars), for the indicated time. (A) Representative Western blots of P-ERK1/2, ERK1/2 and tubulin used as house keeping gene. (B) Relative changes of ERK1/2 phosphorylation and (C) percentage of TUNEL positive INS-1E cells are expressed as means \pm SEM of n=3-4 independent experiments. *p<0.05, ***p<0.001 denotes significance to control culture condition (respective white bar).



observation that PD98059 (10 μ mol/L) inhibited ERK1/2 phosphorylation but accentuated palmitate-induced cell death, without changing basal apoptosis (Fig. 5A-C).

In agreement with our previous observation, TUG-469 (10 μ mol/L) did not augment beta cell death, but significantly reduced palmitate (50 μ mol/L) induced apoptosis (Fig. 5D). The protective effect of TUG-469 was antagonized by PD98059 (10 μ mol/L).

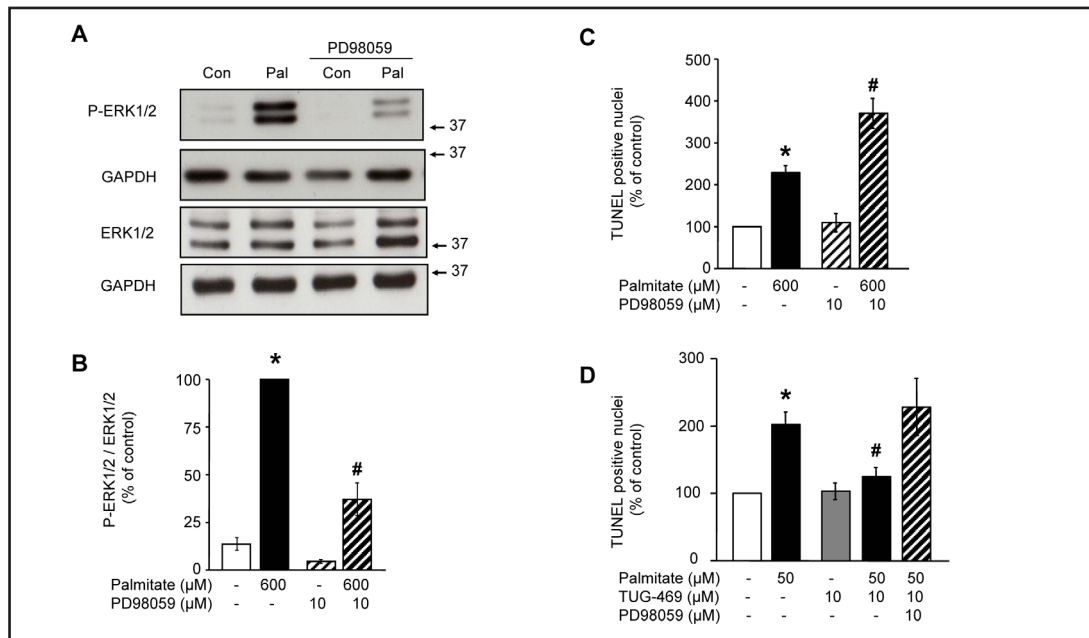


Fig. 5. MEK1 inhibitor accentuates lipotoxicity and reverses the protective effect of TUG-469. INS-E cells cultured under control culture conditions (Con, white bars) and in the presence of palmitate (Pal, 600 μ mol/L, black bars) and PD98059 (10 μ mol/L, hatched bars) as indicated and described under Materials and Methods. (A) Representative Western blots for P-ERK1/2, ERK1/2 and GAPDH as house keeping gene and (B) relative changes of P-ERK1/2 expressed as means \pm SEM of n=3 independent experiments. (C and D) Percentage of TUNEL-positive INS-1E cells expressed as means \pm SEM of (C) n=7 and (D) n=4 independent experiments. *p<0.05 denotes significance to control culture condition (respective white bar), #p<0.05 denotes significance to palmitate (black bar).

In conclusion, palmitate mediated activation of ERK1/2 depends at least in part on GPR40/FFAR1 and is neither crucial for stimulation of insulin secretion nor for induction of apoptosis. In contrast, the results suggest an anti-apoptotic role of ERK1/2 under conditions of lipotoxicity.

Discussion

This study reveals that the specific activation of GPR40/FFAR1 by TUG-469 mimics the stimulation of ERK1/2 induced by palmitate, indicating that GPR40/FFAR1 activation contributes to this effect of palmitate. Intriguingly, the activation of ERK1/2 does not seem to be decisive for GPR40/FFAR1-dependent stimulation of insulin secretion, since the efficient inhibition of ERK1/2 by PD98059 did not affect stimulation of insulin secretion. The most prominent effect of PD98059 in our study was the accentuation of palmitate-induced beta cell death. As PD98059 also antagonized the protective effect of TUG-469 on apoptosis, we propose that the anti-apoptotic effect of GPR40/FFAR1 activation, which we described previously, involves the stimulation of ERK1/2 [3]. A comparable anti-apoptotic role for GPR40/FFAR1 has been suggested previously using the unsaturated fatty acid oleate which also activated ERK1/2 in NIT-1 cells [24]. In contrast to these observations another study associated ERK1/2 phosphorylation with insulin secretion and chronic stimulation of GPR40/FFAR1 with beta cell death [14]. An explanation for the differences could be the use of high concentrations of the MEK inhibitor U0126, which has been found to induce side effects such as increases in the cellular ratios of ADP/ATP and AMP/ATP [25]. This may explain the inhibitory effect of U0126 on secretion. Although high concentrations of

PD98059 have similar unspecific effects, especially in the inhibition of other stress kinases, at the concentration used in our study, PD98059 inhibited ERK1/2 phosphorylation without affecting palmitate-induced phosphorylations of JNK and c-Jun (Fig. 3). Interestingly, the muscarinic M3 receptor, which also couples via Gq to PLC, stimulates ERK1/2 and exerts protective effects in beta cells, supporting our findings [26].

Activation of ERK1/2, especially by glucose, has been linked to stimulation of insulin secretion [14]. In contrast, our study showed that a concentration of PD98059 (10 μ mol/L), which was sufficient to reverse the effect of glucose on ERK1/2 phosphorylation, significantly augmented glucose-induced insulin secretion in INS-1E cells [13]. In Min6 cells glucose-induced insulin secretion was supported at least in part by ERK1/2-dependent phosphorylation of synapsin I [27]. However, in a recent study insulin secretion remained normal in synapsin I deficient beta cells doubting the role of synapsin I in insulin exocytosis [28]. Thus, the role of ERK1/2 in glucose-induced insulin secretion remains elusive.

Furthermore, palmitate-induced phosphorylation of ERK1/2 was not decisive for insulin secretion. PD98059 significantly reduced phosphorylation of ERK1/2 without affecting palmitate-dependent stimulation of insulin secretion. Moreover, basal secretion at 2.8 mmol/L glucose was not stimulated by palmitate, although palmitate induced a strong phosphorylation of ERK1/2 at low glucose.

Of note, the stimulation of ERK1/2 phosphorylation by glucose and palmitate was at least partly additive, hinting at the presence of two distinct pathways which activate ERK1/2. One pathway involves the activation of PKCs and this pathway is activated by PMA, a strong albeit unselective activator of PKCs, but also by palmitate and probably glucose [29-31]. The other pathway involves a stimulation of ERK1/2 through an increase of cytosolic Ca^{2+} , which is the main signalling pathway of glucose [12, 22].

Thus, our observations do not support the idea that ERK1/2 activity is crucial for acute stimulation of insulin secretion by glucose and palmitate. Our study, however, does not exclude that ERK1/2 may exert indirect effects on secretion. ERK1/2 is involved in glucose-dependent regulation of insulin gene transcription and thus supports insulin production [11]. In accordance, in mouse islets and the rat insulinoma cell line INS-1, overexpression of ERK2 increased insulin gene transcription, while a kinase inactive ERK2 mutant inhibited glucose-stimulated transcription, suggesting that ERK2 positively affects insulin production [32]. In contrast, another study using the mouse insulinoma cell line Min6 suggested that glucose-mediated stimulation of the insulin promoter via PDX-1 requires PI3K but not ERK1/2 [33]. Nevertheless, a long term exposure of insulin secreting cells to ERK1/2 inhibitors results in a reduction of insulin content and thus exhaustion of secretion.

In conclusion, GPR40/FFAR1 agonists, besides their beneficial effects on insulin secretion, may exert positive effects on beta cell survival possibly via stimulation of ERK1/2.

Disclosure Statement

The authors declare that there is no duality of interest associated with this study.

Abbreviations

DAPI (4'-6-diamino-2-phenylindole); ERK1/2 / MAPK (extracellular signal-regulated protein kinases 1 and 2); FCS (fetal calf serum); GIP (gastric inhibitory polypeptide); GLP-1 (glucagon-like peptide 1); GPR40/FFAR1 (free fatty acid receptor 1); HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); JNK1/2 (c-Jun N-terminal kinases); KRB (Krebs-Ringer buffered saline); MEK1 / MAPKK1 (mitogen-activated protein kinase kinase 1); PD98059 (MEK1 inhibitor); PFA (paraformaldehyde); PKA (protein kinase A); PKC (protein kinase C); PMA (phorbol 12-myristate 13-acetate); PMSF (phenylmethylsulfonyl fluoride); TUG-469 (FFAR1 agonist); TUNEL (TdT-mediated dUTP-biotin nick end labeling); U0126 (MEK inhibitor).

Acknowledgments

We thank S. Haug (University of Tübingen and IDM, Tübingen and Munich, Germany), D. Neuscheler and E. Metzinger (University of Tübingen, Tübingen, Germany) for skilled technical assistance. MP is a fellow of the International Graduate Program funded by the German Research Foundation (GRK-1302). C-AT was a fellow of the graduate program of the faculty of Medicine at the University Hospital of Tübingen (IKZF). The study was supported by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e. V.).

References

- 1 Latour MG, Alquier T, Oseid E, Tremblay C, Jetton TL, Luo J, Lin DC, Poitout V: GPR40 is necessary but not sufficient for fatty acid stimulation of insulin secretion in vivo. *Diabetes* 2007;56:1087-1094.
- 2 Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M: Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 2003;422:173-176.
- 3 Wagner R, Kaiser G, Gerst F, Christiansen E, Due-Hansen ME, Grundmann M, Machicao F, Peter A, Kostenis E, Ulven T, Fritsche A, Häring HU, Ullrich S: Reevaluation of fatty acid receptor 1 as a drug target for the stimulation of insulin secretion in humans. *Diabetes* 2013;62:2106-2111.
- 4 Natalicchio A, Labarbuta R, Tortosa F, Biondi G, Marrano N, Peschechera A, Carchia E, Orlando MR, Leonardini A, Cignarelli A, Marchetti P, Perrini S, Laviola L, Giorgino F: Exendin-4 protects pancreatic beta cells from palmitate-induced apoptosis by interfering with GPR40 and the MKK4/7 stress kinase signalling pathway. *Diabetologia* 2013;56:2456-2466.
- 5 Wu J, Sun P, Zhang X, Liu H, Jiang H, Zhu W, Wang H: Inhibition of GPR40 protects MIN6 beta cells from palmitate-induced ER stress and apoptosis. *J Cell Biochem* 2012;113:1152-1158.
- 6 Seljeset S, Siehler S: Receptor-specific regulation of ERK1/2 activation by members of the "free fatty acid receptor" family. *J Recept Signal Transduct Res* 2012;32:196-201.
- 7 Ullrich S: IGF-1 and insulin-receptor signalling in insulin secreting cells: from function to survival; in Islam MS (ed.): *The Islets of Langerhans*, Edition 2. *Adv Exp Med Biol Springer* 2014, chapter 23, pp 659-685.
- 8 Frodin M, Sekine N, Roche E, Filloux C, Prentki M, Wollheim CB, VanObberghen E: Glucose, other secretagogues, and nerve growth factor stimulate mitogen-activated protein kinase in the insulin-secreting beta-cell line, INS-1. *J Biol Chem* 1995;270:7882-7889.
- 9 Ehses JA, Pelech SL, Pederson RA, McIntosh CH: Glucose-dependent insulinotropic polypeptide activates the Raf-Mek1/2-ERK1/2 module via a cyclic AMP/cAMP-dependent protein kinase/Rap1-mediated pathway. *J Biol Chem* 2002;277:37088-37097.
- 10 Briaud I, Lingohr MK, Dickson LM, Wrede CE, Rhodes CJ: Differential activation mechanisms of Erk-1/2 and p70(S6K) by glucose in pancreatic beta-cells. *Diabetes* 2003;52:974-983.
- 11 Lawrence M, Shao C, Duan L, McGlynn K, Cobb MH: The protein kinases ERK1/2 and their roles in pancreatic beta cells. *Acta Physiol (Oxf)* 2008;192:11-17.
- 12 Arnette D, Gibson TB, Lawrence MC, January B, Khoo S, McGlynn K, Vanderbilt CA, Cobb MH: Regulation of ERK1 and ERK2 by glucose and peptide hormones in pancreatic beta cells. *J Biol Chem* 2003;278:32517-32525.
- 13 Khoo S, Cobb MH: Activation of mitogen-activating protein kinase by glucose is not required for insulin secretion. *Proc Natl Acad Sci U S A* 1997;94:5599-5604.
- 14 Watson ML, Macrae K, Marley AE, Hundal HS: Chronic effects of palmitate overload on nutrient-induced insulin secretion and autocrine signalling in pancreatic MIN6 beta cells. *PLoS ONE* 2011;6:e25975.
- 15 Cheng Q, Dong W, Qian L, Wu J, Peng Y: Visfatin inhibits apoptosis of pancreatic beta-cell line, MIN6, via the mitogen-activated protein kinase/phosphoinositide 3-kinase pathway. *J Mol Endocrinol* 2011;47:13-21.
- 16 Avram D, Ranta F, Hennige AM, Berchtold S, Hopp S, Häring HU, Lang F, Ullrich S: IGF-1 protects against dexamethasone-induced cell death in insulin secreting INS-1 cells independent of AKT/PKB phosphorylation. *Cell Physiol Biochem* 2008;21:455-462.

- 17 Wijesekara N, Krishnamurthy M, Bhattacharjee A, Suhail A, Sweeney G, Wheeler MB: Adiponectin-induced ERK and Akt phosphorylation protects against pancreatic beta cell apoptosis and increases insulin gene expression and secretion. *J Biol Chem* 2010;285:33623-33631.
- 18 Hammar E, Parnaud G, Bosco D, Perriraz N, Maedler K, Donath M, Rouiller DG, Halban PA: Extracellular matrix protects pancreatic beta-cells against apoptosis: role of short- and long-term signaling pathways. *Diabetes* 2004;53:2034-2041.
- 19 Simon MN, Azevedo-Martins AK, Amanso AM, Carvalho CR, Curi R: Persistent activation of Akt or ERK prevents the toxicity induced by saturated and polyunsaturated fatty acids in RINm5F beta-cells. *Toxicol Int Vitro* 2008;22:1018-1024.
- 20 Lawrence MC, McGlynn K, Naziruddin B, Levy MF, Cobb MH: Differential regulation of CHOP-10/GADD153 gene expression by MAPK signaling in pancreatic beta-cells. *Proc Natl Acad Sci U S A* 2007;104:11518-11525.
- 21 Cunha DA, Hekerman P, Ladriere L, Bazarra-Castro A, Ortis F, Wakeham MC, Moore F, Rasschaert J, Cardozo AK, Bellomo E, Overbergh L, Mathieu C, Lupi R, Hai T, Herchuelz A, Marchetti P, Rutter GA, Eizirik DL, Cnop M: Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *J Cell Sci* 2008;121:2308-2318.
- 22 Maedler K, Stirling J, Sturis J, Zuellig RA, Spinas GA, Arkhammar PO, Mandrup-Poulsen T, Donath MY: Glucose- and interleukin-1beta-induced beta-cell apoptosis requires Ca²⁺ influx and extracellular signal-regulated kinase (ERK) 1/2 activation and is prevented by a sulfonylurea receptor 1/inwardly rectifying K⁺ channel 6.2 (SUR/Kir6.2) selective potassium channel opener in human islets. *Diabetes* 2004;53:1706-1713.
- 23 Ranta F, Avram D, Berchtold S, Düfer M, Drews G, Lang F, Ullrich S: Dexamethasone induces cell death in insulin secreting cells, an effect reversed by Exendin-4. *Diabetes* 2006;55:1380-1390.
- 24 Zhang Y, Xu M, Zhang S, Yan L, Yang C, Lu W, Li Y, Cheng H: The role of G protein-coupled receptor 40 in lipoapoptosis in mouse beta-cell line NIT-1. *J Mol Endocrinol* 2007;38:651-661.
- 25 Dokladda K, Green KA, Pan DA, Hardie DG: PD98059 and U0126 activate AMP-activated protein kinase by increasing the cellular AMP:ATP ratio and not via inhibition of the MAP kinase pathway. *FEBS Lett* 2005;579:236-240.
- 26 Jain S, Ruiz de Azua I, Lu H, White MF, Guettier JM, Wess J: Chronic activation of a designer G(q)-coupled receptor improves beta cell function. *J Clin Invest* 2013;123:1750-1762.
- 27 Longuet C, Broca C, Costes S, Hani EH, Bataille D, Dalle S: Extracellularly regulated kinases 1/2 (p44/42 mitogen-activated protein kinases) phosphorylate synapsin I and regulate insulin secretion in the MIN6 beta-cell line and islets of Langerhans. *Endocrinol* 2005;146:643-654.
- 28 Wendt A, Speidel D, Danielsson A, Esguerra JL, Bogen IL, Walaas SI, Salehi A, Eliasson L: Synapsins I and II are not required for insulin secretion from mouse pancreatic beta-cells. *Endocrinol* 2012;153:2112-2119.
- 29 Marquardt B, Frith D, Stabel S: Signalling from TPA to MAP kinase requires protein kinase C, raf and MEK: reconstitution of the signalling pathway in vitro. *Oncogene* 1994;9:3213-3218.
- 30 Ueda Y, Hirai S, Osada S, Suzuki A, Mizuno K, Ohno S: Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf. *J Biol Chem* 1996;271:23512-23519.
- 31 Hennige AM, Ranta F, Heinzelmann I, Düfer M, Michael D, Braumüller H, Lutz SZ, Lammers R, Drews G, Bosch F, Häring HU, Ullrich S: Overexpression of kinase-negative protein kinase Cdelta in pancreatic beta-cells protects mice from diet-induced glucose intolerance and beta-cell dysfunction. *Diabetes* 2010;59:119-127.
- 32 Khoo S, Griffen SC, Xia Y, Baer RJ, German MS, Cobb MH: Regulation of insulin gene transcription by ERK1 and ERK2 in pancreatic beta cells. *J Biol Chem* 2003;278:32969-32977.
- 33 Rafiq I, da Silva Xavier G, Hooper S, Rutter GA: Glucose-stimulated preproinsulin gene expression and nuclear trans-location of pancreatic duodenum homeobox-1 require activation of phosphatidylinositol 3-kinase but not p38 MAPK/SAPK2. *J Biol Chem* 2000;275:15977-15984.