Available online at www.sciencedirect.com
ScienceDirect
Toxicology in Vitro 22 (2008) 1018–1024
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Persistent activation of Akt or ERK prevents the toxicity induced by saturated and polyunsaturated fatty acids in RINm5F β-cells

Monique Nouailhetas Simon [a], Anna Karenina Azevedo-Martins [b,*], Angélica M. Amanso [a], Carla R.O. Carvalho [a], Rui Curi [a]

[a] *Department of Physiology and Biophysics, Institute of Biomedical Sciences, Brazil*
[b] *School of Arts, Sciences and Humanities, University of São Paulo, Av. Arlindo Bettio, 1000, CEP: 03828-000, Ermelino Matarazzo, São Paulo SP, Brazil*

Received 8 December 2007; accepted 17 February 2008
Available online 26 February 2008

Abstract

The aim of this study was to investigate whether the toxicity of saturated and polyunsaturated fatty acids (PUFA) on RINm5F cells is related to the phosphorylation state of Akt, ERK and PKCδ. The regulation of these kinases was compared in three experimental designs: (a) 4 h-exposure, (b) 4 h-exposure and a subsequent withdrawn of the FA for a 20 h period and (c) 24 h-exposure. Saturated and PUFA were toxic to RINm5F cells even at low concentrations. Also, evidence is provided for a late (*i.e.* the effect only appeared hours after the treatment) and a persistent regulation (*i.e.* maintenance of the effect for several hours) of Akt, ERK and PKCδ phosphorylation by the FA. Late activation of PKCδ seems important for palmitate cytotoxicity. Persistent activation of the survival proteins Akt and ERK by stearate, oleate and arachidonate might play an important role to prevent the toxic effect of posterior PKCδ activation. The results shown may explain why a short-period exposure to FA is not enough to induce cytotoxicity in pancreatic β-cells, since survival pathways are activated. Besides, when this activation is persistent, it may overcome a posterior induction of death pathways.

Keywords: Fatty acids; Akt/PKB; ERK; PKCδ; MAPK; Lipotoxicity; Cell signaling; Long-time regulation

1. Introduction

Short-period exposure of pancreatic β-cells to fatty acids (FA) raises the glucose-stimulated insulin secretion (Crespin et al., 1969; Paolisso et al., 1995). In contrast, extended exposures (over 12 h) of pancreatic islets, primary β-cells and β-cell lineages to some FA (*e.g.* palmitate) impairs insulin secretion, inhibits DNA synthesis and raises the occurrence of apoptosis (Cousin et al., 2001; Eitel et al., 2003; Kharroubi et al., 2004; Lupi et al., 2002; Nerup et al., 1994; Paolisso et al., 1995; Shimabukuro et al., 1998; Wrede et al., 2002).

Diverging with the proposal that only long-chain saturated FA, such as palmitate (PA) and stearate (SA), are toxic (Eitel et al., 2003; Maedler et al., 2001; Welters et al., 2004), we previously showed that exposure for 24 h to long-chain monounsaturated oleate (OA) and polyunsaturated linoleate (LA), γ-linoleneate (LNA) and eicosapentaenoate (EPA) is also cytotoxic, inducing DNA fragmentation in RINm5F β-cells (Azevedo-Martins et al., 2006).

Several authors have demonstrated that FA regulate the phosphorylation state of serine/threonine (Ser/Thr) protein kinases, such as protein kinase B (PKB/Akt), mitogen-activated protein kinases (MAPK) and protein kinase C (PKC). These kinases are involved in survival and apoptotic signaling pathways in pancreatic β-cells (Briaud et al., 2003; Lupi et al., 2002; Shimabukuro et al., 1998; Wang and Brubaker,

Abbreviations: PA, palmitate; SA, stearate; OA, oleate; LA, linolate; LNA, γ-linolenate; AA, arachidonate; EPA, eicosapentaenoate; DHA, docosahexaenoate; FA, fatty acids; PUFA, polyunsaturated fatty acids; Akt/PKB, protein kinase B; ERK, extracellular-regulated kinases; PKCδ, protein kinase C delta; MAPK, mitogen-activated protein kinase; PI, propidium iodide.

* Corresponding author. Tel.: +55 11 3091 8159; fax: +55 11 3091 7285.
E-mail address: karenina@usp.br (A.K. Azevedo-Martins).

0887-2333/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2008.02.012

2002; Wrede et al., 2002, 2003). The regulation of these pathways was proposed as a mechanism for the toxicity of the FA (Mandrup-Poulsen, 2001). Wrede et al. (2002) and Kharroubi et al. (2004) showed that INS-1 β-cells exposed to 0.4 mM OA for 18 h presented reduced Akt phosphorylation and increased occurrence of apoptosis. On the other hand, transfected INS-1 cells with a constitutively active Akt are more resistant against OA-induced apoptosis (Wrede et al., 2002). Wang and Brubaker (2002) confirmed the cell-surviving role of Akt1 (a protein specifically expressed in the islet of Langerhans) *in vivo*. The induction of this protein expression augmented the β-cell mass in db/db mice (Wang and Brubaker, 2002). Some authors reported that the cooperation between Akt and ERK 1/2 (members of the MAPK protein family) seems to be important for the short-term survival of primary pancreatic β-cells against the toxic effect of serum deprivation and interleukin-1 β treatment (Hammar et al., 2004; Wang and Brubaker, 2002). In addition, ERK is important for both proliferation and differentiation of β-cells (Briaud et al., 2003).

In opposition to Akt and ERK, PKCδ is considered a mediator of apoptosis in pancreatic β-cells (Carpenter et al., 2002; Knutson and Hoenig, 1996). In INS-1 β-cells, oleate and palmitate induced a marked activation of PKCδ, along with a reduction of glucose/IGF-1-induced cell proliferation and of Akt phosphorylation (Wrede et al., 2003). In RIN1046-38 β-cells, PA and SA, but not unsaturated FA, induced PKCδ nuclear translocation and its inhibition with rottlerin partially prevented apoptosis induced by these FA (Eitel et al., 2003). Inhibition of PKCδ by rottlerin probably occurs due to a competition between rottlerin and ATP (Gschwendt et al., 1994).

Although the effect of saturated and monounsaturated FA on Ser/Thr protein kinase activity is well documented, information regarding PUFA is still scarce. Also, the long-time effect on Ser/Thr protein kinase phosphorylation of short-period exposure to FA has not yet been investigated in β-cells. Herein, we tested whether PUFA regulate the phosphorylation state of Akt, ERK and PKCδ. We also investigated if a 4 h FA exposure promotes a long-time (24 h) effect on the phosphorylation state of these kinases in RNIm5F cells. Our hypothesis was that in 4 h-exposure to FA, Akt and ERK pathways would be activated and maintained as such after 24 h, keeping the β-cells alive. However, in the 24 h-exposure, the PKCδ pathway would become active, which could then overcome the initial cell-survival signaling activation, leading to cell death. This hypothesis might explain why extended exposures to FA are toxic to β-cells in contrast to short-time exposures.

2. Materials and methods

2.1. Materials

RPMI-1640 medium, HEPES, penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Fatty acids were from Sigma (St. Louis, MO, USA). Propidium iodide was purchased from ICN Biomedicals (Costa Mesa, CA, USA), citrate from Merck (Darmstadt, Germany) and sodium bicarbonate from Labsynth Products (Diadema, SP, Brazil). The antibodies used were: anti-phospho-Akt Ser 473 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-ERK 1/2 Thr 202/Tyr 204 and anti-phospho-PKCδ Ser 643/676 (Cell Signaling Technology-Bioconcept, Allschwil, Switzerland). The ECL Western Blotting Analysis System revealing kit used was from Amersham Biosciences UK Limited (Little Chalfont Buckinghamshere, England). All cell culture material was purchased from Corning (Corning, NY, USA).

2.2. Cell line and culture conditions

The RINm5F cell line is a clone derived from a rat insulinoma (Bhathena et al., 1984). The cells were cultured in RPMI-1640 medium, containing 11 mM glucose and 10% (v/v) fetal calf serum (FCS), supplemented with glutamine (2 mM), HEPES (20 mM), streptomycin (10,000 μg per mL), penicillin (10,000 UI per mL) and sodium bicarbonate (24 mM). Cells were maintained in an incubator (Sanyo, Middlesex, UK), with humidified atmosphere at 37 °C and 5% CO_2.

2.3. Fatty acid treatment

The cells were submitted to three distinct treatments. In the first treatment (4 h experiment), the cells were exposed to 0.1 mM of different FA for 4 h. In the second treatment (4/24 h experiment), the cells were also exposed to 0.1 mM of different FA for 4 h, but after this exposure period, the cells were maintained for more 20 h in medium with no FA addition. At last, in the third treatment (24 h experiment), the cells were exposed to 0.1 mM of different FA for 24 h. Cells were seeded the day before FA treatment in six well plates for the flow cytometric assays (8×10^5 per well) or in 10 mL culture flasks (7.5×10^6) for immunoblotting assays. The FA used were: saturated – palmitate (PA 16:0) and stearate (SA 18:0); monounsaturated – oleate (OA 18:1); and polyunsaturated – ω-6 linoleate (LA C18:2), γ-linolenate (LNA 18:3), arachidonate (AA 20:4), and ω-3 eicosapentaeneate (EPA 20:5) and docosahexaeneate (DHA 22:6). The FA was dissolved in ethanol. Final medium concentration of ethanol did not exceed 0.5%.

2.4. Cell membrane integrity assay

Cell membrane integrity and DNA fragmentation were analyzed by flow cytometry. Cells were centrifuged at 350 g for 15 min at 4 °C and each pellet obtained was resuspended in 500 μL of phosphate-buffered saline (PBS). Afterwards, 50 μL of propidium iodide (PI) solution (50 mg/mL in PBS) were added and the cells were analyzed in a FACSCalibur flow cytometer (Becton Dickinson, San Juan, California). PI is a highly water-soluble fluorescent compound that cannot penetrate in intact membranes and is generally

excluded from viable cells. It binds to nucleic acids by intercalating between the nitrogen bases. Fluorescence was measured using the FL2 channel (Orange-red fluorescence – 585/42 nm). Ten thousand events were analyzed per sample. Cells with PI fluorescence were then evaluated by using the Cell Quest software (Becton Dickinson) (Lima et al., 2002).

2.5. DNA fragmentation assay

Cells were centrifuged at 350 g, for 15 min, at 4 °C and each pellet obtained was gently resuspended in 300 μL hypotonic solution containing 50 μg per mL PI, 0.1% sodium citrate, and 0.1% Triton X-100. The detergents present in solution permeabilize the cells, which promptly incorporate PI into nucleic acids. The cells were then incubated for 24 h at 4 °C. DNA fragmentation was analyzed by flow cytometry after DNA staining with PI according to the method previously described (Nicoletti et al., 1991). Fluorescence was measured and analyzed as described above.

2.6. Immunoblottings

Cells were washed in ice-cold phosphate buffer saline (PBS) solution and collected with a rubber policeman into 2 mL ice-cold Eppendorf tubes. Total protein was extracted using a boiled extraction buffer (100 mM Trizma, 10 mM EDTA, 10% SDS, 10 mM sodium fluoride, 10 mM sodium orthovanadate and 10 mM sodium pyrophosphate), and 70 μg of each sample was separated in 15% SDS-PAGE gel electrophoresis. The proteins were then transferred to nitrocellulose membranes and incubated with specific antibodies for anti-phospho- Ser 473 Akt, anti-phospho- Thr 202/Tyr 204 ERK 1/2 or anti-phospho-Ser 643/676 PKCδ. The membranes were stained with Ponceau solution to assure equal amounts of protein for each sample. Bands were detected using ECL Western Blotting Analysis System revealing kit (Towbin et al., 1979).

2.7. Statistical analysis

Results are expressed as mean ± SEM. The protein kinase phosphorylation values were obtained by densitometric analysis of the staining using Gelplot2 program (Scion Corporation). Flow cytometric results were analyzed using one-way ANOVA followed by Dunnetś or Tukey's test for multiple comparisons and immunoblotting findings were analyzed using student's *t*-test (FA group *versus* control cells). In all cases, Prism analysis program was used for graph preparation and statistical analysis (Graphpad, San Diego, CA, USA).

3. Results

3.1. Toxicity of the fatty acids

Four hours exposure to FA at 0.1 mM did not promote any toxic effect on RINm5F cells, even for the 4/24 h experiment, in which the 24 h response of the cells to 4 h-exposure was examined (Fig. 1). In contrast, 24 h-exposure to PA, LA, LNA and DHA led 21%, 40%, 25% and 8%, respectively, of the cells to death in comparison with the 5% basal mortality on the control group (Fig. 1A). The 24 h-exposure to PA, LA and DHA also increased DNA fragmentation by 60%, 38% and 28%, respectively (Fig. 1B).

3.2. Short-period regulation of Akt, ERK and PKCδ phosphorylation by FA (4 h experiment)

Four hours exposure of RINm5F cells to FA changed the phosphorylation state of cell-survival proteins. AA and DHA raised Akt phosphorylation by 68% and 61% in 4 h, respectively (Fig. 2A), whereas, PA and SA raised ERK phosphorylation by 78% (Fig. 2B). PKCδ phosphorylation was not affected by the FA after 4h-exposure (Fig. 2C).

3.3. Twenty-four hour effect of the 4 h FA exposure on Akt, ERK and PKCδ phosphorylation (4/24 h experiments)

Cells were treated for 4 h with the FA and the long-time effect (observed 20 h later) of this short-period exposure was then investigated. The 68% increase in Akt phosphorylation induced by AA in 4 h was elevated to 95%. In contrast, DHA did not maintain the initial Akt activation seen in the 4 h-exposure (Fig. 3A). In addition, ERK phosphorylation changed in 24 h by short-time exposure to SA, which maintained the initial increase to a minor extent (38%), compared to control cells (Fig. 3B). Hence, the increase induced by AA and SA in Akt and ERK phosphorylation persisted for at least 20 h.

Interestingly, neither SA, OA, LA, LNA nor EPA was able to regulate Akt phosphorylation in 4 h-exposure, but a significant effect was observed 20 h later. SA, OA, LA, LNA and EPA increased Akt phosphorylation by 46%, 76%, 65%, 51% and 122%, respectively, thus evidencing a late effect (Fig. 3A). PA also induced a late increase of PKCδ phosphorylation by 57% (Fig. 3C).

3.4. Twenty-four hour regulation of Akt, ERK and PKCδ phosphorylation by FA (24 h experiments)

Twenty-four hour exposure to LNA and AA increased Akt phosphorylation by 40% and 44%, respectively (Fig. 4A). PA, SA, LA, LNA and AA increased PKCδ phosphorylation by 52%, 46%, 44% and 71%, respectively, in 24 h (Fig. 4C). DHA reduced ERK phosphorylation by 48% (Fig. 4B).

4. Discussion

As we previously showed (Azevedo-Martins et al., 2006), some PUFA (LA, LNA) lead RINm5F cells to death after 24 h treatment, at concentrations $\geqslant$0.1 mM.

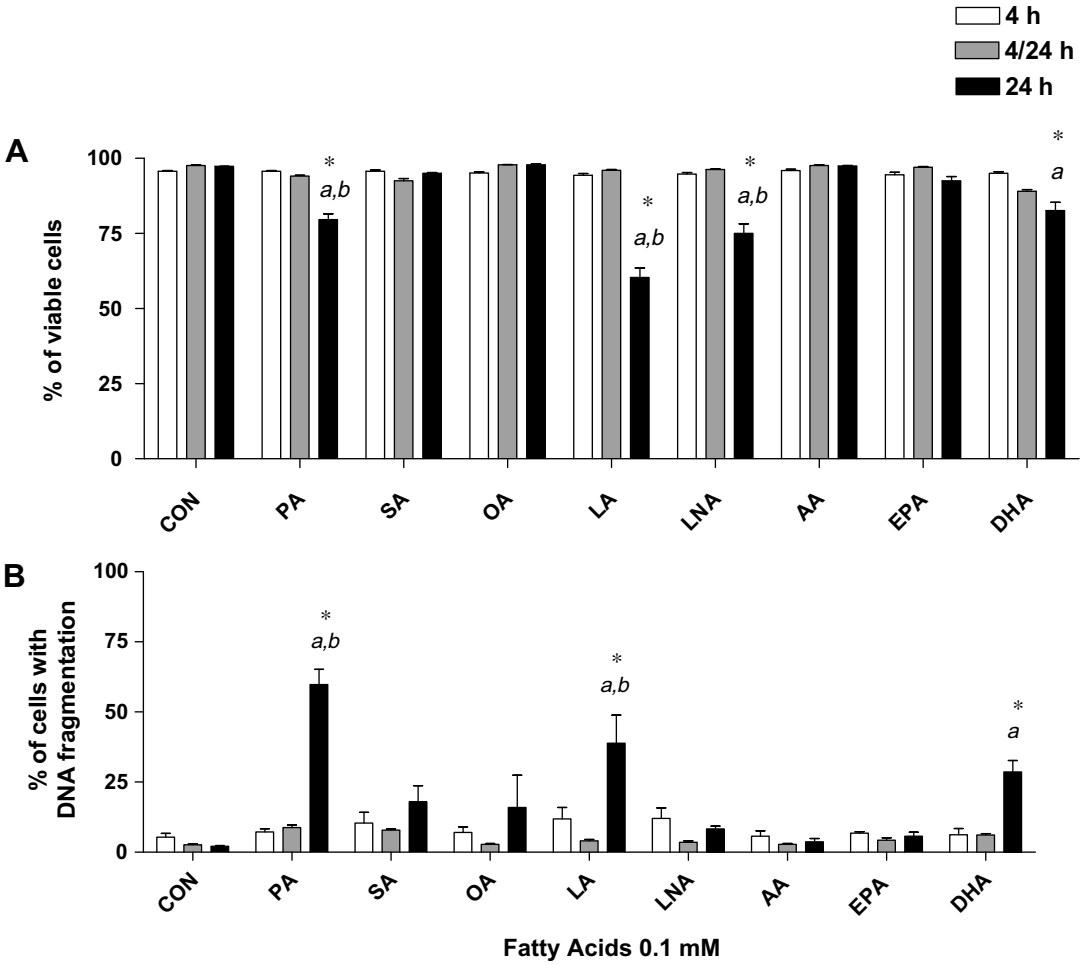

Fig. 1. Fatty acid effect on membrane integrity (A) and DNA fragmentation (B) of insulin-producing RINm5F cells. Cells were incubated for 4 h or 24 h with PA, SA, OA, LA, LNA, AA, EPA or docosahexaenoic DHA at 0.1 mM and analyzed by flow cytometry. White bars – 4 h FA exposure; grey bars – 4 h FA exposure and culture in absence of FA for 20 h; black bars – 24 h FA exposure. Values are presented as mean ± SEM of at least three experiments carried out in triplicate. $^{a}p < 0.01$ FA 4 h compared with FA 24 h-exposure. $^{b}p < 0.01$ FA 4/24 h experiment compared with FA 24 h-exposure (one-way ANOVA plus Tukey's test). $^{*}p < 0.05$ 24 h FA exposure compared with control cells (one-way ANOVA plus Dunnet's test).

This death may occur through necrosis, apoptosis or both. The 0.1 mM concentration of most FA used in our study is within the human blood physiological range (Tiez, 1994). Herein, we demonstrated that FA present toxic effects on pancreatic β-cells even at low concentrations, provided that the time exposure is long enough, as also previously observed (Azevedo-Martins et al., 2006).

FA at 0.1 mM concentration regulated Ser/Thr protein kinase phosphorylation in all periods of exposure. PA presented a more pronounced effect on DNA fragmentation then on decrease of cell membrane integrity, indicating that its toxic effect is not a consequence of physical injury of the cell membrane (as observed for LNA), but mainly due to activation of death pathways in the β-cell. In addition, PA did not activate Akt in any period of incubation, but induced a late increase in PKCδ phosphorylation (*i.e.* a 24 h effect of 4 h-exposure seen in 4/24 h experiment) and sustained it up to 24 h, what might be related to its great toxicity. Therefore, it seems that PKCδ is important for PA toxicity, as pointed out by others (Eitel et al., 2003; Wrede et al., 2003).

PKCδ might be involved in the LA and LNA cytotoxicity, since it was activated by these FA in the 24 h-exposure. Nonetheless, differently from PA, these FA did not promote a late activation of PKCδ, indicating that a 4 h-exposure of the cells to LA and LNA is not sufficient to induce PKCδ phosphorylation. Although the toxicity of the three FA seems to depend on PKCδ, the activation of this protein kinase did not cause inhibition of Akt or ERK phosphorylation as expected. The PKCδ pathway may not necessarily be associated with Akt or ERK. Eitel et al. (2003) reported a nucleus translocation of PKCδ induced by PA and SA in RIN1046-38 cells. The kinase caused laminin B disassembly, due to its direct phosphorylation. This phenomenon could enable caspases to enter the nucleus and reach their sites of cleavage inducing apoptosis (Eitel et al., 2003).

LA and LNA increased Akt phosphorylation as a 24 h effect of 4 h-exposure. However, this increase does not characterize a persistent Akt activation, since no effect on this protein was seen in 4 h experiment, and thus did not prevent PKCδ toxic effects. Activation of Akt is not an

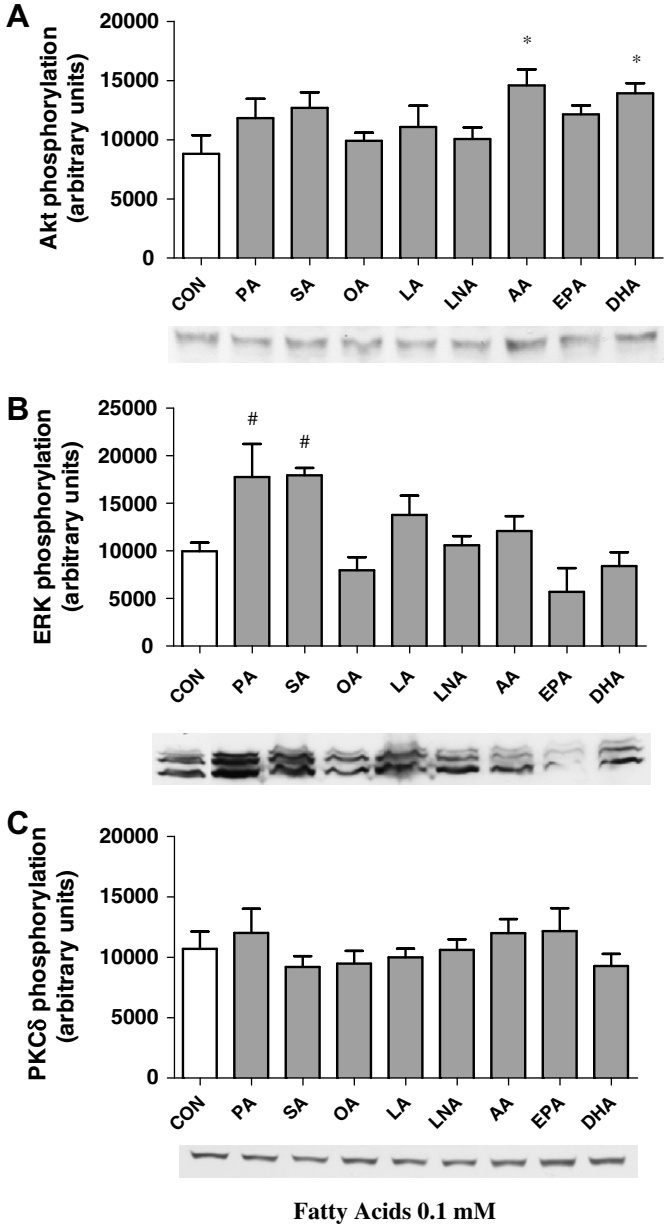

Fig. 2. Short-period regulation of Akt, ERK and PKCδ phosphorylation by FA in insulin-producing RINm5F cells. Cells were incubated with PA, SA, OA, LA, LNA, AA, EPA or DHA at 0.1 mM for 4 h and immunoblotting was promptly performed for (A) Akt, (B) ERK or (C) PKCδ. Values are presented as means ± SEM of six experiments. $^{*}p < 0.05$ and $^{\#}p < 0.01$ (FA exposure compared to CON cells – student's *t*-test).

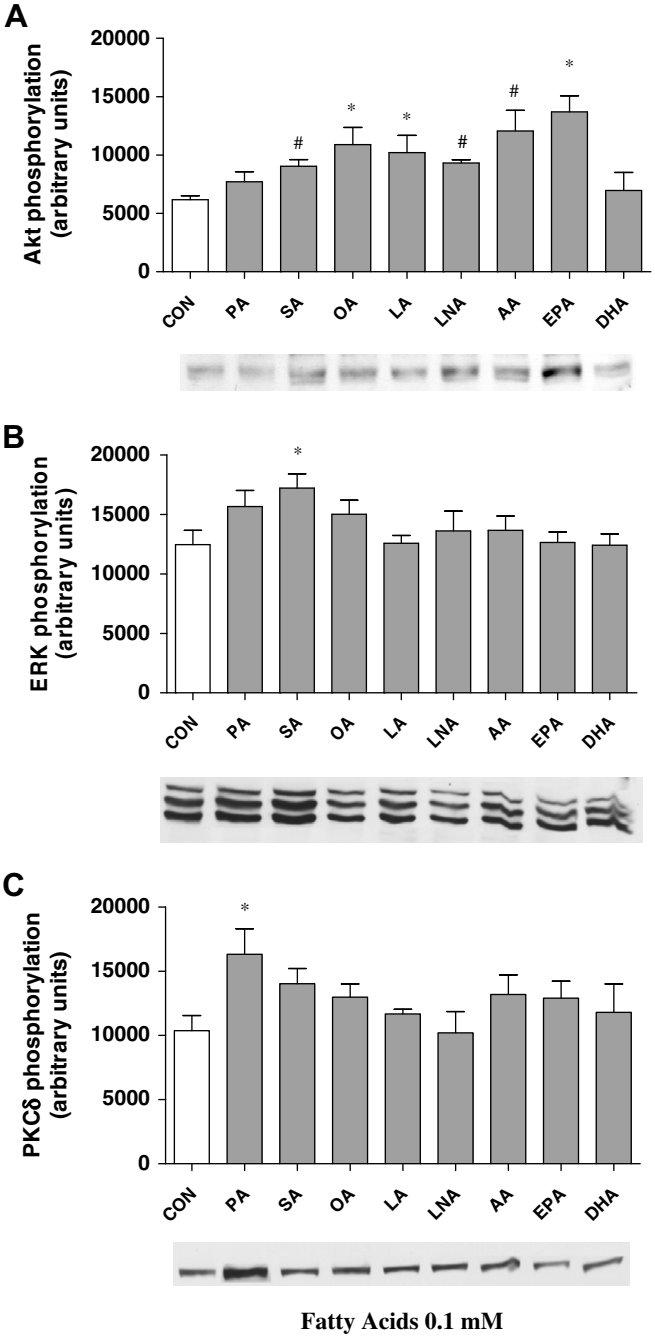

Fig. 3. Long-term effect of short-period FA exposure on Akt, ERK and PKCδ phosphorylation in insulin-producing RINm5F cells. Cells were incubated with PA, SA, OA, LA, LNA, AA, EPA or DHA at 0.1 mM for 4 h and cultured for more 20 h in medium with no FA. Immunoblotting was performed for (A) Akt, (B) ERK or (C) PKCδ. Values are presented as means ± SEM of at least five experiments. $^{*}p < 0.05$ and $^{\#}p < 0.01$ (FA exposure compared to CON cells – student's *t*-test).

often reported response for β-cells exposed to FA. However, Higa et al. (2006) described a dose-dependent increase of Akt phosphorylation induced by exposure to PA for 24 h. The authors hypothesized that Akt presents a bidirectional signaling effect, promoting toxicity when exposed to high concentrations of FA, but leading to survival at low levels. However, in their study, no other proteins were concomitantly analyzed (Higa et al., 2006). We believe that Akt does not induce cytoxicity. The persistent activation of Akt seen herein, induced by low FA concentration and short-period exposure, appears to prevent toxic effects.

Distinctly from the findings of Wrede et al. (2002, 2003) and Kharroubi et al. (2004), OA was not toxic to RINm5F cells. However, the concentration used in our study was four times lower (0.1 mM) than the one employed by these

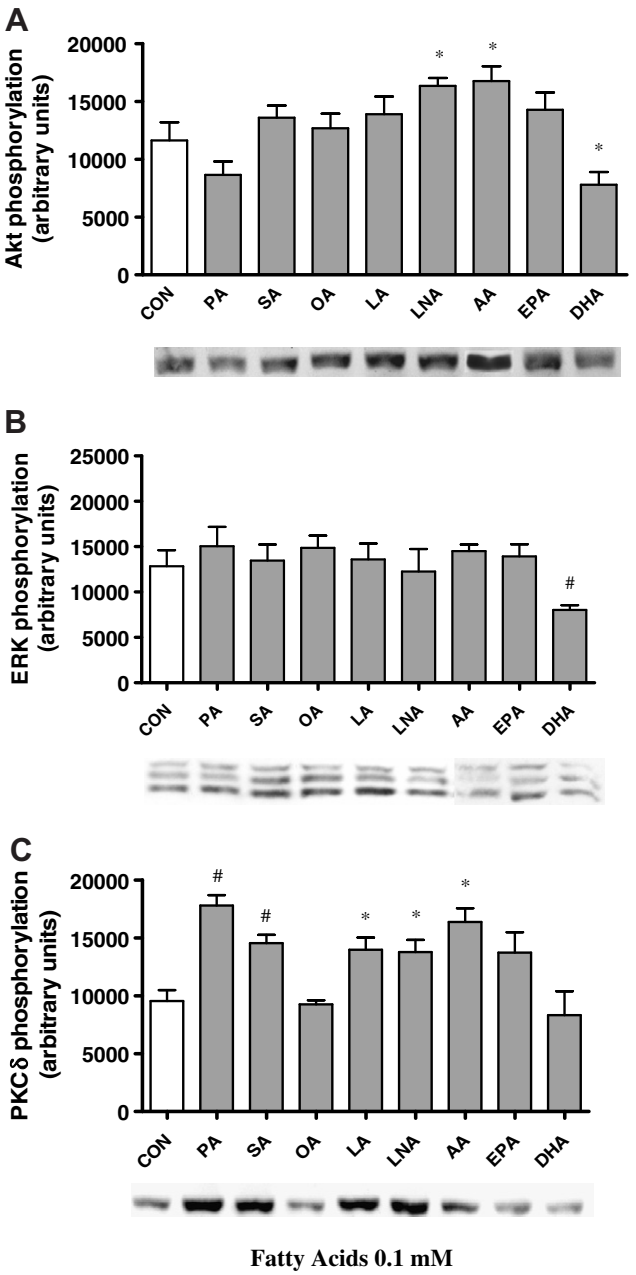

Fig. 4. Long-period regulation of Akt, ERK and PKCδ phosphorylation by FA in insulin-producing RINm5F cells. Cells were incubated with PA, SA, OA, LA, LNA, AA, EPA or DHA at 0.1 mM for 24 h and immunoblotting was performed for (A) Akt, (B) ERK or (C) PKCδ. Values are presented as mean ± SEM of at least five experiments. $^{*}p < 0.05$ and $^{\#}p < 0.01$ (FA exposure compared to CON untreated cells – student's *t*-test).

authors (0.4 mM). In fact, the low concentration of OA allowed the activation of Akt. SA, LA, LNA and EPA also promoted a late increase of Akt phosphorylation, as seen in the 4/24 h experiment. This late activation of Akt might induce anti-apoptotic effects by phosphorylation of GSK3α, Bcl-2 family members or other proteins involved in cell-survival (Dickson and Rhodes, 2004; Elghazi et al., 2006; Lingohr et al., 2002; Woodgett, 2005).

The late effect on Akt phosphorylation may have occurred through enhancement of Akt synthesis or through inhibition of phosphatase activity. These events might occur in a gradual manner, therefore, delaying the manifestation of the effect. FA can affect protein synthesis but only negative effects were described so far. FA reduces protein synthesis by phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α) (Cnop et al., 2007). On the other hand, the α isoform of PH-domain leucine-rich repeat phosphatase (PHLPP) dephosphorylates the serine residue of the regulatory C-terminal domain of Akt (Gao et al., 2005) and its gradual inhibition may result in the late FA effect.

Although DHA activated Akt in the short-period treatment, this effect did not persist. In fact, DHA inhibited Akt phosphorylation in 24 h experiment. So, DHA toxicity was probably related to inhibition of Akt and ERK phosphorylation in the 24 h-exposure. The pathway involved in the DHA toxicity might be different from the ones involved in PA, LA and LNA toxicity. AA also activated Akt phosphorylation after 4 h treatment but, differently from DHA, this effect persisted and it was still enhanced in the 4/24 h experiment. ERK phosphorylation was also sustained by SA (it raised after 4 h and persisted up to 24 h). Considering that AA and SA were not toxic for RINm5F cells but had activated PKCδ in the 24 h-exposure, the persistent activation of survival kinases may be important to protect the pancreatic β-cells against the cytotoxicity expected to occur by increased PKCδ phosphorylation.

At low concentrations and short-period exposure to FA, activation of survival proteins (AKT and ERK) seems to prevail over the death-inducing pathways. On the other hand, when cells are exposed to extended periods, PKCδ activation promotes toxicity in the absence of a persistent activation of Akt or ERK. In conclusion, these findings might explain why a short-period exposure is not enough to induce cytotoxicity in pancreatic β-cells, since survival pathways are activated. Besides, when this activation is persistent, it may overcome a posterior induction of death pathways.

Acknowledgements

The authors wish to thank to G. de Souza, J.R. Mendonça and E.P. Portiolli for technical assistance. Special thanks devoted to Dr. Sandro Massao Hirabara for his help with immunoblotting assays. This research was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

References

Azevedo-Martins, A.K., Monteiro, A.P., Lima, C.L., Lenzen, S., Curi, R., 2006. Fatty acid-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells. Toxicology In Vitro 20, 1106–1113.

Bhathena, S.J., Awoke, S., Voyles, N.R., Wilkins, S.D., Recant, L., Oie, H.K., Gazdar, A.F., 1984. Insulin, glucagon, and somatostatin secretion by cultured rat islet cell tumor and its clones. Proceedings of the Society for Experimental Biology and Medicine 175, 35–38.

Briaud, I., Lingohr, M.K., Dickson, L.M., Wrede, C.E., Rhodes, C.J., 2003. Differential activation mechanisms of ERK-1/2 and p70(S6K) by glucose in pancreatic beta-cells. Diabetes 52, 974–983.

Carpenter, L., Cordery, D., Biden, T.J., 2002. Inhibition of protein kinase C delta protects rat INS-1 cells against interleukin-1beta and streptozotocin-induced apoptosis. Diabetes 51, 317–324.

Cnop, M., Ladriere, L., Hekerman, P., Ortis, F., Cardozo, A.K., Dogusan, Z., Flamez, D., Boyce, M., Yuan, J., Eizirik, D.L., 2007. Selective inhibition of eukaryotic translation initiation factor 2 alpha dephosphorylation potentiates fatty acid-induced endoplasmic reticulum stress and causes pancreatic beta-cell dysfunction and apoptosis. Journal of Biological Chemistry 282, 3989–3997.

Cousin, S.P., Hugl, S.R., Wrede, C.E., Kajio, H., Myers Jr., M.G., Rhodes, C.J., 2001. Free fatty acid-induced inhibition of glucose and insulin-like growth factor I-induced deoxyribonucleic acid synthesis in the pancreatic beta-cell line INS-1. Endocrinology 142, 229–240.

Crespin, S.R., Greenough 3rd, W.B., Steinberg, D., 1969. Stimulation of insulin secretion by infusion of free fatty acids. The Journal of Clinical Investigation 48, 1934–1943.

Dickson, L.M., Rhodes, C.J., 2004. Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt?. American Journal of Physiology – Endocrinology and Metabolism 287 E192–E198.

Eitel, K., Staiger, H., Rieger, J., Mischak, H., Brandhorst, H., Brendel, M.D., Bretzel, R.G., Haring, H.U., Kellerer, M., 2003. Protein kinase C delta activation and translocation to the nucleus are required for fatty acid-induced apoptosis of insulin-secreting cells. Diabetes 52, 991–997.

Elghazi, L., Balcazar, N., Bernal-Mizrachi, E., 2006. Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. The International Journal of Biochemistry & Cell Biology 38, 157–163.

Gao, T., Furnari, F., Newton, A.C., 2005. PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Molecular Cell 18, 13–24.

Gschwendt, M., Muller, H.J., Kielbassa, K., Zang, R., Kittstein, W., Rincke, G., Marks, F., 1994. Rottlerin: a novel protein kinase inhibitor. Biochemical and Biophysical Research Communications 199, 93–98.

Hammar, E., Parnaud, G., Bosco, D., Perriraz, N., Maedler, K., Donath, M., Rouiller, D.G., Halban, P.A., 2004. Extracellular matrix protects pancreatic beta-cells against apoptosis: role of short- and long-term signaling pathways. Diabetes 53, 2034–2041.

Higa, M., Shimabukuro, M., Shimajiri, Y., Takasu, N., Shinjyo, T., Inaba, T., 2006. Protein kinase B/Akt signalling is required for palmitate-induced beta-cell lipotoxicity. Diabetes, Obesity and Metabolism 8, 228–233.

Kharroubi, I., Ladriere, L., Cardozo, A.K., Dogusan, Z., Cnop, M., Eizirik, D.L., 2004. Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress. Endocrinology 145, 5087–5096.

Knutson, K.L., Hoenig, M., 1996. Regulation of distinct pools of protein kinase C delta in beta cells. Journal of Cellular Biochemistry 60, 130–138.

Lima, T.M., Kanunfre, C.C., Pompeia, C., Verlengia, R., Curi, R., 2002. Ranking the toxicity of fatty acids on Jurkat and Raji cells by flow cytometric analysis. Toxicology In Vitro 16, 741–747.

Lingohr, M.K., Buettner, R., Rhodes, C.J., 2002. Pancreatic beta-cell growth and survival – a role in obesity-linked type 2 diabetes? Trends in Molecular Medicine 8, 375–384.

Lupi, R., Dotta, F., Marselli, L., Del Guerra, S., Masini, M., Santangelo, C., Patane, G., Boggi, U., Piro, S., Anello, M., Bergamini, E., Mosca, F., Di Mario, U., Del Prato, S., Marchetti, P., 2002. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 51, 1437–1442.

Mandrup-Poulsen, T., 2001. Beta-cell apoptosis: stimuli and signaling. Diabetes 50 (Suppl. 1), S58–S63.

Maedler, K., Spinas, G.A., Dyntar, D., Moritz, W., Kaiser, N., Donath, M.Y., 2001. Distinct effects of saturated and monounsaturated fatty acids on beta-cell turnover and function. Diabetes 50, 69–76.

Nerup, J., Mandrup-Poulsen, T., Helqvist, S., Andersen, H.U., Pociot, F., Reimers, J.I., Cuartero, B.G., Karlsen, A.E., Bjerre, U., Lorenzen, T., 1994. On the pathogenesis of IDDM. Diabetologia 37 (Suppl. 2), S82–S89.

Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani, F., Riccardi, C., 1991. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. Journal of Immunological Methods 139, 271–279.

Paolisso, G., Gambardella, A., Amato, L., Tortoriello, R., D'Amore, A., Varricchio, M., D'Onofrio, F., 1995. Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. Diabetologia 38, 1295–1299.

Shimabukuro, M., Zhou, Y.T., Levi, M., Unger, R.H., 1998. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proceedings of the National Academy of Science USA 95, 2498–2502.

Tiez, N.W., 1994. Textbook of Clinical Chemistry, second ed. Wiley, Philadelphia, p. 2188.

Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Science USA 76, 4350–4354.

Wang, Q., Brubaker, P.L., 2002. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 45, 1263–1273.

Welters, H.J., Tadayyon, M., Scarpello, J.H., Smith, S.A., Morgan, N.G., 2004. Mono-unsaturated fatty acids protect against beta-cell apoptosis induced by saturated fatty acids, serum withdrawal or cytokine exposure. Federation of European Biological Sciences Letters 560, 103–108.

Wrede, C.E., Dickson, L.M., Lingohr, M.K., Briaud, I., Rhodes, C.J., 2002. Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). Journal of Biological Chemistry 277, 49676–49684.

Wrede, C.E., Dickson, L.M., Lingohr, M.K., Briaud, I., Rhodes, C.J., 2003. Fatty acid and phorbol ester-mediated interference of mitogenic signaling via novel protein kinase C isoforms in pancreatic beta-cells (INS-1). Journal of Molecular Endocrinology 30, 271–286.

Woodgett, J.R., 2005. Recent advances in the protein kinase B signaling pathway. Current Opinion in Cell Biology 17, 150–157.