ACADEMIC PRESS

Biochemical and Biophysical Research Communications 296 (2002) 189–193

BBRC

www.academicpress.com

Fatty acids potentiate interleukin-1β toxicity in the β-cell line INS-1E

M. Aarnes, S. Schønberg, and V. Grill*

Department of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, Trondheim, Norway

Received 26 June 2002

Abstract

Evidence for "lipotoxicity," i.e., negative effects of fatty acids (FA) on pancreatic β-cells is incomplete. Here, we tested whether non-toxic concentrations of FA potentiate toxic effects of interleukin-1β (IL-1β). Culture of INS-1E clonal β-cells for 2–6 days with 70 μM docosahexaenoic acid (DHA), eicosapentaenoic acid, arachidonic acid, 0.1 mM linoleic acid, or 0.1–0.2 mM oleic acid exerted no or minor negative effects on cell viability (MTT assay). Viability was reduced by 0.5 ng/ml IL-1β. All tested FA significantly aggravated this effect after 6 days of culture. IL-1β also exerted negative effects on cellular insulin content and DHA and oleic acid aggravated these effects. L-NAME, an inhibitor of constitutive nitric oxide (NO) synthase, reduced the negative effects of IL-1β per se but did not abolish the potentiating effects of FA. Conclusion: FA potentiate toxic effects of IL-1β on β-cells by mechanisms that include NO-independent ones.

Keywords: Fatty acids; β-Cells; INS-1; Interleukin-1β; Cytokines; Insulin biosynthesis

Interactions of fatty acids (FA) with insulin producing β cells are complex. It was documented many years ago that acute exposures to FA in vivo and in vitro stimulate insulin secretion to a moderate degree [1,2]. It is also well known that FA serve as important nutrients in β-cells [3]. More recently, it was found that longer-term exposures to elevated FA in vivo and in vitro could inhibit glucose-induced insulin secretion [4–6] and insulin biosynthesis [5,7]. Also, elevated FA have been linked under some experimental conditions to β-cell apoptosis [8] and reduction of β-cell mass.

The question arises to which extent other factors, such as those present in vivo, interact during long-term exposure to FA to exert negative effects on β-cell function and survival. This important aspect of FA influence has largely been unexplored, except for the effect of concomitant hyperglycemia [9]. A recent study indicated that oleate and palmitate up-regulate pro-inflammatory genes [10]. This information prompted us to investigate whether FA could modulate the toxicity of interleukin-1β (IL-1β) towards β-cells. To test for interactions with this cytokine would be of particular interest because IL-1β plays an important role in the destruction of β-cells in type 1 diabetes [11]. To address the question of interaction we purposely used low concentrations of both FA and IL-1β to optimize conditions for detecting interactions. We have used a variant of the clonal β-cell line, INS-1 cells of rat origin with documented sensitivity to glucose [12] and IL-1β [13].

Materials and methods

Materials. Oleic and linoleic acids, interleukin-1-β (IL-1β), *N*-ω-nitro-L-arginine methyl ester (L-NAME) and BSA were obtained from Sigma, St. Louis, MO. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) were obtained from Cayman Chemical, Ann Arbor, MI. All fatty acids were obtained or prepared in stock ethanol solutions. Control conditions with the corresponding concentrations of ethanol (1%) were run in each experiment.

Cell culture. INS-1E cells were a gift from Dr. Claes Wollheim, Geneva, Switzerland. Cells were grown in monolayer cultures in RPMI-1640 medium containing 11 mmol/L glucose supplemented with 10 mmol/L HEPES, 10% fetal calf serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 50 μmol/L mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37 °C in a humidified

* Corresponding author. Present address. Department of Internal Medicine, University Hospital of Trondheim, N-7006 Trondheim, Norway. Fax. +47-73-86-75-46.

E-mail address: valdemar.grill@medisin.ntnu.no (V. Grill).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00819-7

(5% CO_2, 95% air) atmosphere. Cells were seeded 7 days before use in 75 cm^2 flasks at a density of 3.0×10^6 cells per flask. They were subcultured once a week to new flasks. The passage number of the INS-1E cells was between 61 and 75 in the present experiments.

Cell viability experiments. Cells were seeded in individual wells, 2×10^4 cells in 100 µl in each well in RPMI medium, and cultured for 2 days at 37 °C. The medium was then replaced with test medium containing appropriate additions. Microtiter plates containing 96 wells were cultured for 2, 3 or 6 days after which MTT assays [14] were performed as outlined below.

Insulin contents of INS-1E cells. Cells were seeded in 1-ml wells containing 2.5×10^5 cells and cultured with RPMI medium for 48 h after which the appropriate test additions were made. Subsequently the cells were cultured for 2 or 6 days after which they were washed twice with 1 ml Krebs–Henseleit bicarbonate (KHB) buffer. Then, 200 µl acid ethanol (0.18 M HCl in 95% ethanol) was added to the cells. The tubes containing cells and acid ethanol were kept overnight in a refrigerator and then stored at −20 °C for subsequent insulin measurements. Insulin was measured by RIA, using an anti-porcine insulin antibody (raised in the Department of Endocrinology, Karolinska Hospital, Stockholm).

MTT assay. A stock solution of 3-(4,5,-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml) was prepared in phosphate-buffered saline (PBS), diluted in RPMI medium and added to cell-containing wells at a concentration of 0.5 mg/ml, 100 µl per well, after first removing additives and medium. The plates were then incubated for 3 h at 37 °C in 5% CO_2. Fifty µl liquid was aspirated, leaving a total volume of 50 µl in each well. One-hundred µl of 2-propanol supplemented with HCl (3.3 ml/L) was added to solubilize the MTT formazan. The plates were then placed on a mechanical shaker for 20–60 min at room temperature for complete solubilization. Absorbency was measured on a multiscan plus reader with a 588-nm wavelength filter. All experiments were performed at least three times, each time with 16 parallels.

Presentation of results. Results are presented as means ± SE. Significance testing was carried out using the non-parametric Mann–Whitney test for unpaired samples.

Results

Effect on β-cell viability by different FA

The effect of addition of different FA on cell viability was tested after 2, 3, and 6 days of culture (Fig. 1). Only

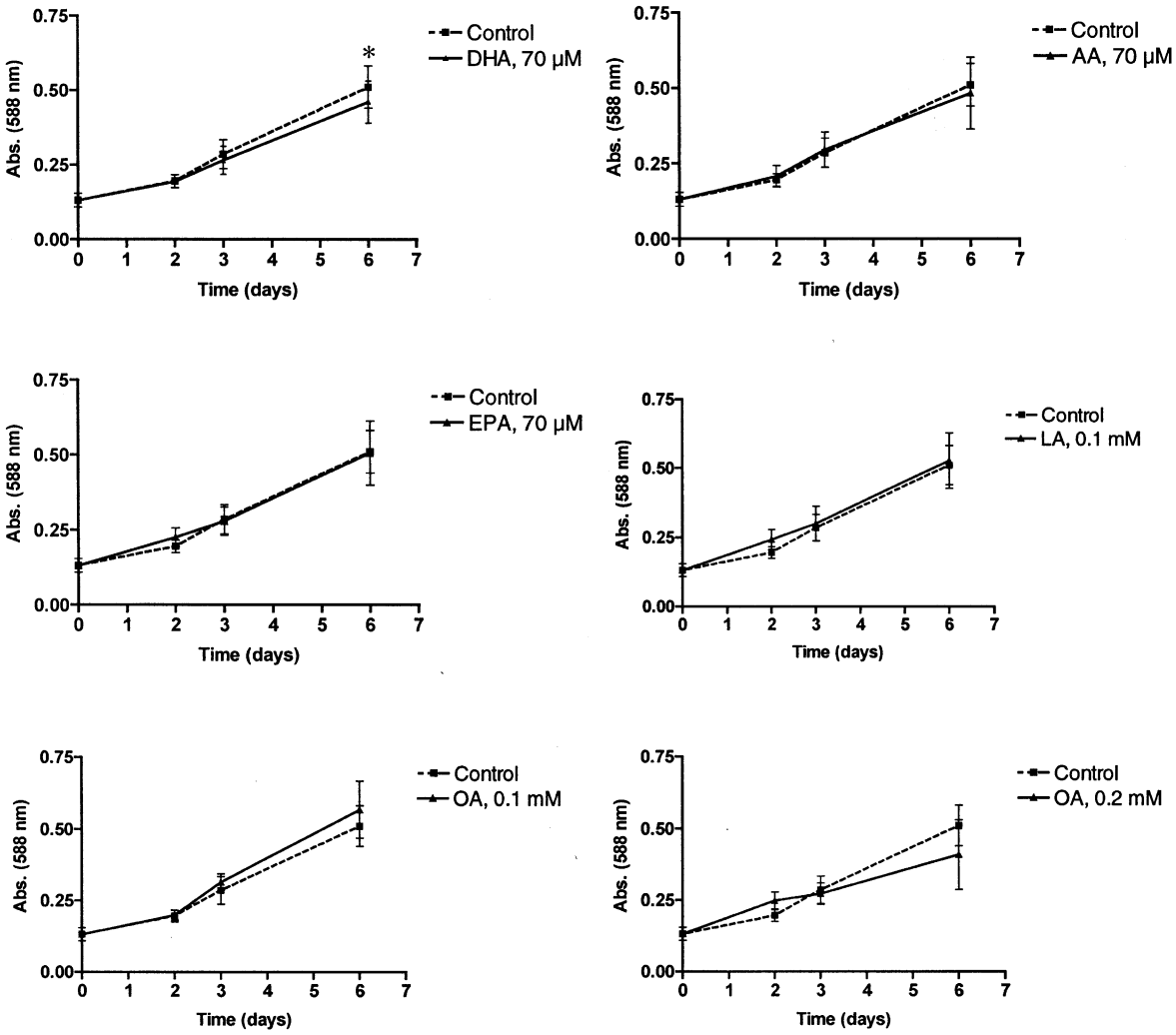

Fig. 1. Effects of different FA (docosahexanoic acid, DHA, eicosapentaenoic acid, EPA, arachidonic acid, AA, linoleic acid, LA, and oleic acid, OA on the viability of INS-1E cells during culture for 2, 3, and 6 days. The results represent means ± SE for three separate experiments, each containing 16 parallels. *, $p < 0.05$ for effects of FA.

after 6 days, there was a significant negative effect of DHA and a non-significant effect of 0.2 mM oleic acid ($p < 0.07$). The ethanol concentration of control media did not per se diminish the MTT measurements. Thus, the absorbency at 588 nm in the assay at 2, 3, and 6 days of culture was 0.178 ± 0.032, 0.255 ± 0.047, and 0.462 ± 0.066 for cultures without ethanol and 0.196 ± 0.021, 0.285 ± 0.048, and 0.510 ± 0.072 for cultures with ethanol.

Effects on cell viability of different FA in combination with IL-1β

IL-1β (0.5 ng/ml) reduced the MTT-assessed viability of cells by 44% after 2 days and by 24% after 6 days of culture (Fig. 2). This effect of IL-1β was enhanced by all FA tested after 6 days of culture. Notably, EPA, AA, and linoleic acid failed by themselves to reduce the cell viability (Fig. 1) but still potentiated the negative effect of IL-1β (Fig. 2).

Effects of FA and IL-1β on insulin contents

Two days of culture failed to affect insulin contents in cells from wells cultured with DHA or oleate (Table 1) whereas IL-1β significantly decreased insulin contents. Insulin contents were reduced further by co-culture of IL-1β with either DHA or oleate.

After 6 days, the insulin contents of DHA and oleate-cultured wells were still comparable with the control

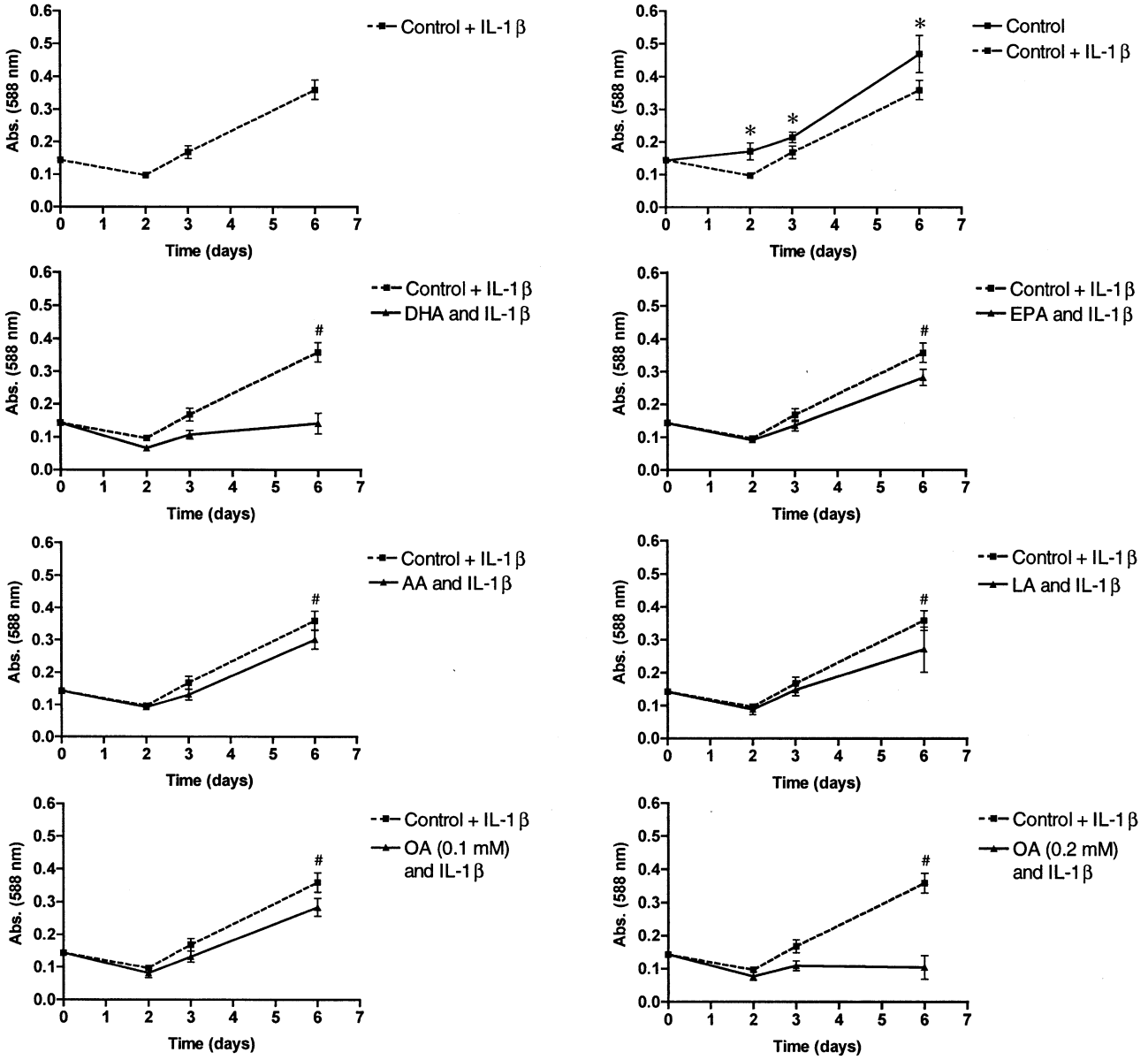

Fig. 2. Modulating influence of fatty acids on IL-1β induced effects on cell viability. Abbreviations and symbols as for Fig. 1. Means ± SE of three experiments, each with 16 parallels. *, $p < 0.05$ for effects of IL-1β; #, $p < 0.05$ for effects of FA.

Table 1
Effects of FA and IL-1β on cellular insulin contents[a]

Additions	Insulin (mU/well) Culture (days)	
	2	6
None (ethanol)	91.4 ± 10.0	130.2 ± 6.7
DHA	117.6 ± 8.4	124.2 ± 8.1
Oleic acid, 0.2 mM	93.1 ± 9.4	157.7 ± 21.2
IL-1β	43.7 ± 6.2[b]	152.6 ± 37.5
DHA + IL-1β	26.3 ± 2.4[c]	64.5 ± 17.9[c]
Oleic acid + IL-1β	16.5 ± 2.3[c]	4.5 ± 1.3[c]

[a] Means ± SE of six experiments.
[b] $p < 0.05$ for effects of IL-1β.
[c] $p < 0.05$ for effects of FA vs. IL-1β.

ones (Table 1), as were also wells with IL-1β-cultured cells. However, culture with a combination of IL-1β and either DHA or oleate led to a marked decrease in insulin contents.

Effects of L-NAME on FA-induced potentiation of IL-1β toxicity

Toxicity of IL-1β is partly coupled to the production of nitric oxide [15]. In line herewith, inhibitors of NO exert protective effects on IL-1β toxicity. Such an effect was confirmed here using the cNOS inhibitor L-NAME. Culture with 2.0 mm L-NAME had no effect per se but improved the cell survival during exposure to IL-1β (Table 2). However, the addition of L-NAME did not abolish the potentiation effect of oleic acid on IL-1β toxicity (Table 2).

Discussion

We demonstrate, to our knowledge for the first time, a marked potentiation by FA of IL-1β toxicity. Especially notable is the fact that most of the FA at the concentrations tested did not produce measurable negative effects by themselves, yet were able to potentiate the negative effects of IL-1β. Potentiation was apparent both with regard to cell viability as measured by the MTT assay and with regard to cellular insulin contents. Our findings are compatible with observations in islets of Zucker-diabetic rats in which depletion of islet triglyceride stores by leptin and troglitazone reduced IL-1β toxicity [16].

We observe that the negative effects of IL-1β per se were stronger after 2 days than after 6 days of culture. Lesser toxicity with time could be due to selection of cells relatively resistant to IL-1β. Such selection in INS-1 cells was recently reported [17], albeit after a longer time of culture with IL-1β. It was probably favored by using a low concentration of IL-1β (0.5 ng/ml), a concentration that was identical to that used in the present experiments. Resistance to IL-1β was associated with up-regulation of transcription factors, such as STAT-1 [18], and down-regulation of genes such as that for the IL-1β receptor [17]. Resistance to IL-1β could also be due to the induction with time of genes coding for antioxidants such as that of superoxide dismutase [19].

The potentiating effects of FA were more prominent after 6 than after 2 days of exposure during culture. This could be due to an offsetting influence on one or more of the protective genes mentioned. In that context, it is of interest that oleate and palmitate up-regulate the gene expression for an IL-1β receptor in another clonal β cell line, Min6 [10]. Alternatively or additionally FA may diminish DNA replication [20], which in turn could diminish the evolution of IL-1β resistant cells. As to a role for increased NO production by FA, the cNOS inhibitor L-NAME did not abolish the FA-induced potentiating effects on IL-1β-induced toxicity. This observation suggests that factors other than NO production participate in the potentiating effect of FA. This notion is supported by the failure of oleate to increase the nitrite production in β-cells, despite observed cytotoxicity [21], thus, questioning the proposed [8,16] role of FA-induced NO production for FA-induced apoptosis.

Table 2
Effects of L-NAME on FA and IL-1β induced decreases in cell viability[a]

Additions	Culture (days)			
	2		6	
	Absorbance[b]	% Inhibition	Absorbance[b]	% Inhibition
Solvent (ethanol)	279 ± 37		586 ± 43	
L-NAME	270 ± 36		589 ± 38	
IL-1β	185 ± 26	34	347 ± 35	41
IL-1β + L-NAME	239 ± 40	14	422 ± 25[c]	28
Oleic acid, 0.2 mM	340 ± 48		597 ± 27	
Oleic acid + L-NAME	351 ± 36		659 ± 49	
Oleic acid + IL-1β	172 ± 14	50	165 ± 35	72
Oleic acid + IL-1β + L-NAME	206 ± 17	40	355 ± 51[c]	46

[a] Means ± SE of three experiments.
[b] 588 nM × 10^3.
[c] $p < 0.05$ for effect of L-NAME.

In our experiments, DHA and oleic acid did not decrease cellular insulin contents. Consequently, a possible negative effect on insulin biosynthesis could not be discerned under the present conditions, in contrast to previous studies[5,7]. The comparatively low concentrations of FA used here could explain differences vs. the inhibitory effects found in the previous studies.

It is obvious that the present results with clonal β-cells of rat origin cannot be extrapolated to in vivo situations in human. Nevertheless, the notion that FA can potentiate negative effects of IL-1β is of potential interest for the etiology of type 1 diabetes. In a general sense, the notion that negative effects of FA are contingent upon other factors is one that should be pursued in further studies.

Acknowledgments

This study was supported by the Norwegian Research Council (Grant No. 136139/310), by the Norwegian Diabetes Association and by the NovoNordisk Foundation.

References

[1] S. Crespin, W. Greenough, D. Steinberg, Stimulation of insulin secretion by infusion of free fatty acids, J. Clin. Invest. 48 (1969) 1934–1943.
[2] W. Malaisse, F. Malaisse-Lagae, Stimulation of insulin secretion by non-carbohydrate metabolites, J. Lab. Clin. Med. 72 (1968) 438–448.
[3] W. Malaisse, L. Best, S. Kawazu, F. Malaisse-Lagae, A. Sener, The stimulus-secretion coupling of glucose-induced insulin release: fuel metabolism in islets deprived of exogenous nutrients, Arch. Biochem. Biophys. 224 (1983) 102–110.
[4] Y. Sako, V. Grill, A 48 h lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and β-cell oxidation through a process coupled to fatty acid oxidation, Endocrinology 127 (1990) 1580–1589.
[5] Y-P. Zhou, V. Grill, Long term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle, J. Clin. Invest. 93 (1994) 870–876.
[6] Y. Lee, H. Hiroshi, M. Ohneda, J. Johnson, D. McGarry, R. Unger, β Cell lipotoxicity in the pathogenesis of non-insulindependent diabetes mellitus of obese rats, impairment in adipocyte-β-cell relationships, Proc. Natl. Acad. Sci. USA 91 (1994) 10878–10882.
[7] C. Bollheimer, R. Skelly, M. Chester, D. McGarry, C. Rhodes, Chronic exposure to free fatty acid reduces pancreatic β cell insulin content by increased basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation, J. Clin. Invest. 101 (1998) 1094–1110.
[8] M. Shimabukuro, Y-T. Zhou, M. Levi, R. Unger, Fatty acid-induced apoptosis: a link between obesity and diabetes, Proc. Natl. Acad. Sci. USA 95 (1998) 2498–3202.
[9] T. Briaud, C. Rouault, G. Reach, V. Poitout, Inhibition of insulin gene expression by long term exposure of pancreatic β cells to palmitate is dependent on the presence of a stimulatory glucose concentration, Metabolism 49 (2000) 532–536.
[10] A. Busch, D. Cordery, G. Denyer, T. Biden, Expression profiling of palmitate and oleate-regulated genes provide novel insights into the effects of chronic lipid exposure on pancreatic β-cell function, Diabetes 51 (2002) 977–987.
[11] T. Mandrup-Poulsen, The role of interleukin-1 in the pathogenesis of IDDM, Diabetologia 39 (1996) 1005–1029.
[12] M. Asfari, D. Janjic, P. Meda, G. Li, P. Halban, C. Wollheim, Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines, Endocrinology 130 (1992) 167–178.
[13] D. Janjic, M. Asfari, Effects of cytokines on rat insulinoma INS-1 cells, J. Endocrinol. 132 (1992) 67–76.
[14] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[15] D. Eizirik, E. Cagliero, A. Bjorklund, N. Welsh, Interleukin-1β induces the expression of an isoform of nitric oxide synthase in insulin-producing cells, which is similar to that observed in activated macrophages, FEBS Lett. 308 (1992) 249–252.
[16] M. Shimabukuro, K. Koyama, Y. Lee, R. Unger, Leptin- or troglitazone-induced lipopenia protects islets from interleukin 1β cytotoxicity, J. Clin. Invest. 100 (1997) 1750–1754.
[17] G. Chen, H. Hohmeier, R. Gasa, V. Tran, C. Newgard, Selection of insulinoma cell lines with resistance to interleukin-1β- and γ-interferon-induced cytotoxicity, Diabetes 49 (2000) 562–570.
[18] G. Chen, H. Hohmeier, C. Newgaard, Expression of the transcription factor STAT-1α in insulinoma cells protects against cytotoxic effects of multiple cytokines, J. Biol. Chem. 276 (2001) 766–772.
[19] A. Cardozo, M. Kruhøffer, R. Leeman, T. Ørntoft, D. Eizirik, Identification of novel cytokine-induced genes in pancreatic β cells by high density oligonucleotide arrays, Diabetes 50 (2001) 909–920.
[20] S. Cousin, S. Hugl, C. Wrede, H. Kajio, M. Myers Jr., C. Rhodes, Free fatty acid-induced inhibition of glucose and insulin-like growth factor I-induced deoxyribonucleic acid synthesis in the pancreatic β-cell line INS-1, Endocrinology 142 (2001) 229–240.
[21] M. Cnoop, J.C. Hannaert, A. Hoorens, D. Eizirik, D. Pipeleers, Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation, Diabetes 50 (2001) 1771–1777.