Research Article

Islet Brain 1 Protects Insulin Producing Cells against Lipotoxicity

Saška Brajkovic,1,2 Mourad Ferdaoussi,1,2,3 Valérie Pawlowski,2,4 Hélène Ezanno,2 Valérie Plaisance,2 Erik Zmuda,5 Tsonwin Hai,5 Jean-Sébastien Annicotte,2 Gérard Waeber,1 and Amar Abderrahmani2

1Service of Internal Medicine, Centre Hospitalier Universitaire Vaudois and University of Lausanne, 1011 Lausanne, Switzerland
2University of Lille, European Genomic Institute for Diabetes (EGID) FR 3508, UMR CNRS 8199, Faculty of Medicine West, 1 place de Verdun, 59045 Lille, France
3Department of Pharmacology and the Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada
4University of Lille, EGID FR 3508, Department of Endocrine Surgery, Lille University Hospital, UMR INSERM 1190, Lille, France
5Department of Molecular and Cellular Biochemistry, Ohio State University, 1060 Carmack Road, Columbus, OH, USA

Correspondence should be addressed to Amar Abderrahmani; amar.abderrahmani@univ-lille2.fr

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Chronic intake of saturated free fatty acids is associated with diabetes and may contribute to the impairment of functional beta cell mass. Mitogen activated protein kinase 8 interacting protein 1 also called islet brain 1 (IB1) is a candidate gene for diabetes that is required for beta cell survival and glucose-induced insulin secretion (GSIS). In this study we investigated whether IB1 expression is required for preserving beta cell survival and function in response to palmitate. Chronic exposure of MIN6 and isolated rat islets cells to palmitate led to reduction of the IB1 mRNA and protein content. Diminution of IB1 mRNA and protein level relied on the inducible cAMP early repressor activity and proteasome-mediated degradation, respectively. Suppression of IB1 level mimicked the harmful effects of palmitate on the beta cell survival and GSIS. Conversely, ectopic expression of IB1 counteracted the deleterious effects of palmitate on the beta cell survival and insulin secretion. These findings highlight the importance in preserving the IB1 content for protecting beta cell against lipotoxicity in diabetes.

1. Introduction

Type 2 diabetes is one of the major health challenges of the 21st century. The disease arises when beta cells produce insufficient insulin to meet the increased hormone demand, caused by insulin resistance or growth of tissues such as liver, muscle, and adipose tissues. Although genome-wide association studies revealed a genetic contribution in the etiology of the disease [1], the environmental risks factors are very likely the most prominent cause of beta cell decline in the vast majority of cases [2]. Lifestyle changes such as lack of physical activity together with excessive adiposity contribute to chronic elevation of the circulating plasma saturated free fatty acids (FFAs). Numerous studies have highlighted that chronic exposure to elevated levels of FFAs, in particular palmitate, is detrimental by promoting insulin resistance and beta cell dysfunction [3]. The beta cell failure elicited by palmitate includes a defect in their secretory capacity to respond to glucose and a loss of beta cell mass by apoptosis [4–8]. These diabetogenic effects of palmitate are in part achieved by modulating the expression and activity of proapoptotic and antiapoptotic proteins [3, 9–20].

The mitogen activated protein kinase 8 interacting protein 1, also named islet brain 1 (IB1) or c-Jun N Terminal Kinase- (JNK-) interacting protein 1 (JIP1), is mainly expressed in islet beta cells and is one of the key antiapoptotic factors of this cell type [21–24]. Reduction of the IB1 content in insulin producing and islets cell increases apoptosis [25–27]. A wealth of data reports the diminution of IB1 level, as a major mechanism through which inflammatory cytokines...
cause beta cell apoptosis [22, 23, 25–29]. Some studies have ascribed the protective role of IBI to the regulation of JNK pathway, although the exact mechanism of this regulation is still unclear [30, 31]. Reduction of IBI expression may activate phosphorylation of JNK targets [30]. A mutation within the coding region of this gene has been associated with a rare and monogenic form of diabetes and induces beta cell death in vitro [23]. Conversely, overexpression of IBI renders cells more resistant to apoptosis induced by cytokines [22, 23, 26, 27, 29]. Moreover, induction of IBI is a major target of the glucagon-like peptide 1 mimetics for preventing beta cell death [26]. However, the role of IBI in the context of lipotoxicity has not been reported thus far. In this report, we demonstrated the roles of IBI in palmitate-induced beta cell death and function and described the regulation of IBI by palmitate at both the transcriptional and posttranslational levels.

2. Material and Methods

2.1. Materials. Palmitate (sodium salts) was obtained from Sigma-Aldrich (St. Louis, MO). The saturated fatty acid was coupled to bovine serum albumin by 1% agitation at 37°C and freshly prepared for each experiment [32]. This procedure yielded BSA-coupled fatty acids in a molar ratio of 5:1. The MG132 compound was purchased from Sigma-Aldrich (St. Louis, MO). The antibodies against IBI, mSIN3, and c/ebpβ were obtained from Santa Cruz Biotechnology (CA, USA).

2.2. Islets Preparation, Cell Culture, and Transfection. Rat islets were isolated from the pancreas of Sprague-Dawley rats (male, at body weight of 250–350 g) by ductal injection of collagenase. The purification and culture of islets were conducted as described [29]. The mouse insulin-secreting cell line MIN6 was cultured in DMEM glutaxam medium (Invitrogen, Carlsbad, CA) supplemented with 15% FCS, 50 U/mL penicillin, 50 µg/mL streptomycin, and 70 µM β-mercaptoethanol [33]. The rat insulin-secreting cell line INS-1E was maintained in RPMI 1640 medium supplemented with 10% FCS, 1 mM Sodium Pyruvate, 50 µM β-mercaptoethanol, and 10 mM Hepes [26]. The plasmid encoding HA-IB1-WT and siRNA duplexes directed against IBI (si-IB1), GFP (si-GFP), or ICER (siICER) were previously described [26, 34]. Plasmids or the siRNA duplexes were introduced using the Lipofectamine 2000 (Invitrogen AG) exactly as described [26].

2.3. Measurement of Insulin Secretion. The MIN6 cells (10^5) were plated in 24-well dishes. Two days after transfection, cells were washed twice with PBS. Thereafter, cells were preincubated in KRHB buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH2PO4, 0.5 mM MgSO4, 1.5 mM CaCl2, 2 mM NaHCO3, 10 mM HEPES, 0.1% bovine serum albumin, and pH 7.4) containing 2 mM glucose for 1 hour. Afterward medium was changed with KRHB buffer containing 2 mM glucose corresponding to basal state or with 20 mM glucose for an additional 45 minutes. Insulin secretion was measured by EIA (SPI-BIO) according to manufactured protocol.

2.4. Western Blotting. The cells were scrapped in the PBS and lysed by using a NP-40 lysis buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, and 1% NP-40) supplemented with antiproteases and antiphosphatases (Roche). 25–40 µg of total protein extracts was separated on 10% SDS-polyacrylamide gel and electrically blotted to nitrocellulose membrane. The proteins were detected using a buffer containing 0.1% Tween 20 and 5% milk and incubated overnight at 4°C with specific primary antibodies and were visualized with IRDye 800 or IRDye700 (Rockland) as secondary antibodies. Quantification was realized using the Odyssey Infrared Imaging System (Li-COR).

2.5. Reverse Transcription Coupled with Quantitative PCR (RT-qPCR). Total RNA was extracted using guanidium thiocyanate-phenol-chloroform RNA purification method. Reverse transcription was performed as described [34]. Real-time quantitative-PCR assays were carried out on the Bio-Rad MyiQ Real-Time PCR Detection System using iQ SYBR Green Supermix (Bio-Rad) as the amplification system with 100 nM primers and 2 µL of template (RT product) in 20 µL of PCR volume and annealing temperature of 59°C. Primers sequences were as follows: mouse Ibi, sense 5'-ACA AGG GCA ATG ATG TCC TC-3' and antisense 5'-TTT ATT TCC CTT GGC CTC C-3'; mouse housekeeping ribosomal protein, large P0 (Rplp0), sense 5'-ACCTCTTCCTTCCAGGGCTTT-3' and antisense 5'-CCACCTTGTCTCTCAGTCTTT-3'; mouse Bcl2, sense 5'-CTCCCGATTTCGCAAGTT-3' and antisense 5'-TCTACTTCCCTCCGCAA-3'.

3. Results

3.1. Reduction of Ibi Content in MIN6 Cells by Palmitate Relieved on the Transcriptional Repressor ICER and Proteasome-Mediated Degradation. A large number of reports have confirmed the adverse effects of palmitate on function and survival of isolated islets and different insulin-secreting cells including MIN6 cells [11, 13, 15, 19]. For this reason we chose to monitor the Ibi mRNA level in MIN6 cells and isolated rat islets that were cultured with palmitate. RT-qPCR showed reduction of Ibi mRNA in islet and MIN6 cells cultured with palmitate for 48 and 72 hrs (Figure 1(a)). Because palmitate modulates the activity of several transcription factors [11], we tested the hypothesis that the decreased Ibi mRNA levels resulted from reduced transcriptional activity of its promoter. The human proximal IBI promoter contains several key elements that promote expression and regulation of the gene in beta cell [35]. A 731bp fragment of the proximal promoter has been cloned upstream of the luciferase reporter (IBI-luc) and is highly active in insulin producing cells [24]. As previously observed, luciferase activity of the IBI-luc construct was 20–25-fold higher than the promoterless control vector in MIN6 cells (Figure 1(b)). This activity was reduced by twofold when the cells were cultured in the presence of palmitate (Figure 1(b)). The IBI promoter contains a CAMP response element (CRE) [26]. This element binds the inducible cAMP early repressor (ICER) [26], an antagonist of the CRE-binding protein (CREB). ICER
Figure 1: Effect of palmitate on the $I_b1$ mRNA level. (a) Quantification of $I_b1$ mRNA level by RT-qPCR from MIN6 cells (open bar) and isolated rat islets (grey bar) cultured with 0.5 mM palmitate or BSA (−) at different indicated times. (b) Assessment of $I_b1$ transcriptional activity in MIN6 cells cultured with palmitate. Cells were transiently transfected with a luciferase reporter construct driven by the 731 bp fragment of the human $MAPK8IP1$ promoter (IB1luc). Palmitate was added to the medium 24 hrs after transfection and luciferase activity was measured 48 hrs later. To test the role of ICER, IB1luc was cotransfected together with duplexes of control small interfering RNA (siGFP, open bar) or siRNA directed specifically against ICER (siICER, filled bar). The data are expressed as fold increase over the control vector pGL3basic and are the mean ± SEM of three independent experiments. (c) Role of ICER in the drop of $I_b1$ mRNA induced by palmitate. The $I_b1$ mRNA was measured by RT-qPCR in MIN6 cells that were transfected with duplexes of either siGFP (open bar) or siICER (filled bar). After transfection, (24 hrs) the cells were cultured with BSA (ctrl) or 0.5 mM palmitate for additional 48 hrs. The results were normalized against $Rplp0$ and the expression levels from cells cultured with BSA were set to 100%. Data are the mean ± SEM of 3 independent experiments ($^{**}P < 0.01$; $^*P < 0.05$).
expression rises up in beta cells incubated with palmitate [36]. We have previously demonstrated that overexpression of ICER represses the promoter activity of IB1-luc in beta cells [26]. To investigate whether ICER links palmitate to reduced Ib1 mRNA levels, we transfected MIN6 cells with siRNA directed against ICER (siICER) that we previously validated in beta cells [28, 34, 37]. Interestingly, silencing of ICER restored IB1 luc activity and Ib1 mRNA levels in the presence of palmitate (Figures 1(b) and 1(c)), supporting a role for ICER in the reduction of Ib1 expression induced by palmitate. Activating transcription factor 3 (ATF3), which also binds to the CRE site, is a potent repressor of gene expression induced by palmitate in beta cells [35, 38, 39]. However, the Ib1 expression was neither reduced in cells in which Atf3 was overexpressed (see supplementary Figure 1a in Supplementary Material available online at http://dx.doi.org/10.1155/2016/9158562) nor increased in islets cells from atf3 knockout mice, thus ruling out a role for Atf3 in the loss of Ib1 mRNA caused by the saturated fatty acid (supplementary Figure 1b). Two Ib1 isoforms, one corresponding to the full length protein and one from the use of an alternative promoter [26], were detected in MIN6 cells by immunoblotting experiments (Figure 2(a)) [26, 27]. A significant reduction in Ib1 protein levels was apparent after 24 hrs treatment of cells with palmitate (Figure 2(a)). These results were confirmed in isolated rat islets cultured with palmitate for 24 hrs (Figure 2(b)). Palmitate hampers insulin expression, secretion, and cell survival by inducing the expression of C/EBPβ [11]. Interestingly we observed that the decreased Ib1 protein level was concomitant with the increased C/EBPβ protein levels (Figures 2(a) and 2(b)). Chronic hyperglycemia potentiates the harmful effects of palmitate [9] in INS-1E cells but not in MIN6 or isolated human islets [40]. To determine whether the effects of palmitate were potentiated by glucose, Ib1 protein levels were quantified in INS-1E cells cultured with palmitate in the presence of low or high glucose concentration (5 or 20 mmol/L glucose, resp.). A similar reduction of Ib1 by palmitate was observed upon low or high glucose concentration (Figure 2(c)), indicating that palmitate decreases the expression of high glucose concentration. Since decreased Ib1 protein levels occurred prior to the decrease of its mRNA levels (Figures 1(a) and 2(a)), this suggests that the reduction of Ib1 mRNA is not the only mechanism affecting its protein content. The fatty acid affects beta cell survival and function through ER stress dependent pathways [3, 15–17]. Palmitate impairs Ca2+ influx to ER by affecting
Figure 3: Effect of the proteasome inhibitor MG132 on the decrease of Ib1 content caused by palmitate. Ib1 content was measured from MIN6 cells exposed to (a) 0.5 mM palmitate or (b) 1 μM thapsigargin (thaps) for 48 hrs in the presence or absence of 1 μM of the proteasome inhibitor MG132. The Ib1 level was normalized against the mSin3a. The figure shows the result of a representative experiment out of three. The results are expressed as the mean ± SEM of three independent experiments (**P < 0.01; *P < 0.05).

3.2. Overexpression of Ib1 Counteracts the Deleterious Effects of Palmitate on Glucose-Induced Insulin Secretion and Cell Survival. Ib1 is required for glucose-induced insulin secretion and cell survival [23, 26, 43]. We investigated whether the decreased Ib1 level contributes to palmitate-induced cell death by ectopically expressing Ib1. As shown in Figures 4(a) and 4(b), IB1 partially rescued the cells as evidenced by the reduction in apoptotic cell number and increase in the mRNA level of Bcl2, an antiapoptotic gene. Conversely, silencing of Ib1 using a previously validated siRNA [26] potentiated the effect of palmitate on cell death (Figure 4(a)) with a concomitant increase of the Bcl2 mRNA (Figure 4(b)). We next investigated whether the reduction of Ib1 by palmitate could contribute to defective glucose-induced insulin secretion. As previously shown [43], silencing of Ib1 in Min6 cells reduced glucose-induced insulin secretion (Figure 5), which was exacerbated in the presence of palmitate (Figure 5). Transient ectopic expression of Ib1 can overcome proteasome-mediated degradation of Ib1 elicited by cytokines [22, 23, 27, 44]. It has been previously shown that Ib1 overexpressing beta cells are more resistant to apoptosis [22, 23, 27, 44]. Interestingly and in line with these observations, Ib1 overexpressing Min6 cells improved their glucose-induced insulin secretion when chronically exposed to palmitate (Figure 5).

4. Discussion

Evidence for the potential diabetogenic role of palmitate by afflicting beta cell function and survival has been provided...
Figure 4: Role of Ibi in apoptosis induced by palmitate. (a) MIN6 cells were transfected with the siRNA duplex directed against Ibi (siIbi) or control siRNA (siGFP, Ctrl) or the plasmids coding for the wild type HA-tagged Ibi (Ibi). For scoring death, 0.5 mM palmitate (filled bars) or BSA (Ctrl, open bars) was added to the cells medium 24 hrs after transfection. The rate of apoptosis was scored by counting pycnotic nuclei in cells exposed for 48 hrs to palmitate. Results are expressed as mean ± SEM of 3 independent experiments (∗P < 0.05; ∗∗P < 0.01; ∗∗∗P < 0.001). (b) For the quantification of Bcl2, total RNA from transfected cells with siGFP, siIbi, and Ibi was prepared and subjected to qPCR. The levels of Bcl2 were compared in cells incubated with BSA (open bars) and 0.5 mM palmitate (filled bars) for 48 hrs. The mRNA were normalised against Rplp0 and those of the control cells were set to 100%. Data are the mean ± SEM of five independent experiments (∗P < 0.05).

by a plethora of data from in vitro and in vivo experiments [3, 7, 12, 41]. Palmitate decreases beta cell survival by promoting apoptosis [19]. Reduction of the antiapoptotic Ibi expression is a major mechanism eliciting beta cell apoptosis in response to cytokines and oxidized LDL [25, 26, 28, 29]. However, its role in lipotoxicity has not been reported. In this report, we show that palmitate decreases Ibi gene expression at both the transcriptional and posttranslational levels. At the transcriptional level, the effect is dependent on the transcriptional repressor ICER, since silencing of ICER dampened the ability of palmitate to reduce Ibi mRNA. Although the level of ATF3, another transcriptional repressor, is increased by palmitate [39], our data indicate that ATF3 is not necessary for palmitate to repress Ibi gene expression. Interestingly, our data showed that diminution of Ibi protein content occurs earlier than the drop of Ibi mRNA and this was via a proteasome-mediated pathway. Overexpression of Ibi protects beta cell against apoptosis triggered by cytokines [22, 25]. In line with this protective effect we observed that ectopic expression of Ibi prevented the deleterious effect of palmitate on cell survival.

Beside its antiapoptotic role, Ibi regulates glucose-induced insulin secretion [21, 23, 43]. Consistent with this metabolic function, inactivation of Ibi alters insulin secretion stimulated by glucose [43]. Herein we confirmed that silencing of Ibi mimics the effect of palmitate on insulin secretion. Moreover, ectopic expression of Ibi partially restored glucose-induced insulin secretion in response to palmitate, indicating that exogenous expression of Ibi compensates for the decrease of Ibi content caused by palmitate. Ibi is described as a scaffold protein that assembles the kinases involved in the JNK activation; however, paradoxically its function is to inhibit JNK activity [22, 25, 26, 29, 31]. JNK activation often (but not always) precedes JNK activity. The c-Jun transcription factor is a JNK target that is deemed to couple JNK activation to apoptosis [45]. JNK phosphorylates c-Jun and this could lead to apoptosis [45, 46]. Independent studies have shown that Ibi level may be required for inhibiting phosphorylation of c-Jun [25, 27]. Overexpression of Ibi level reduces phosphorylation of c-Jun caused by cytokines in islets and insulin producing cells [25, 27].

JNK pathway is activated in response to several diabetogenic stresses including oxidized LDL and cytokines [26, 29]. Increased JNK activity is a key mechanism coupling palmitate to beta cell dysfunction and ultimately cell death [3, 19], and inhibition of JNK activity alleviates the adverse
effects of palmitate [3, 19]. Thus, our finding that palmitate reduces Ibi expression may provide a potential mechanism for palmitate to increase JNK activity. There are three JNK isoforms identified so far [47]. All of them are present in beta cells [48]. There are growing studies pointing to divergent roles in JNK isoforms in beta cells. While JNK2 seems to be proapoptotic, JNK1 and JNK3 are antiapoptotic [48–50]. Therefore, further analyses are required to determine whether and how Ibi may regulate each of the JNK isoforms. Understanding such regulation will permit us to elucidate the mechanism through which Ibi preserves beta cell against the harmful effects caused by palmitate. Inhibition of the JNK pathway has been proposed as a potential therapeutic way for treating beta cell failure in type 2 diabetes and some efforts are currently maintained to identify novel JNK inhibitors [51]. Future investigation of Ibi activity may help in finding out novel targets exploitable in the design of next innovative therapies of T2D.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution
Saška Brajkovic, Mourad Ferdaoussi, and Valérie Pawlowski equally contributed to the work.

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