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Role of cytosolic and calcium independent phospholipases A_2 in insulin secretion impairment of INS-1E cells infected by *S. aureus*

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ABSTRACT

Cytosolic PLA_2 ($cPLA_2$) and Ca^{2+}-independent PLA_2 ($iPLA_2$) play a significant role in insulin β-cells secretion. Bacterial infections may be responsible of the onset of diabetes. The mechanism by which *Staphylococcus aureus* infection of INS-1 cells alters glucose-induced insulin secretion has been examined. After acute infection, insulin secretion and PLA_2 activities significantly increased. Moreover, increased expressions of phospho-$cPLA_2$, phospho-PKCα and phospho-ERK 1/2 were observed. Chronic infection causes a decrease in insulin release and a significant increase of $iPLA_2$ and COX-2 protein expression. Moreover, insulin secretion in infected cells could be restored using specific *si*RNAs against $iPLA_2$ isoform and specific COX-2 inhibitor.

1. Introduction

Phospholipases A_2 (PLA_2) are a large family of enzymes ubiquitously expressed that catalyze the breakdown of glycerophospholipids, releasing arachidonic acid (AA): cytosolic PLA_2 ($cPLA_2$), Ca^{2+}-independent PLA_2 ($iPLA_2$), and Ca^{2+}-dependent secretory PLA_2 ($sPLA_2$) differ from each other in terms of substrate specificity and Ca^{2+}-requirement [1]. The $cPLA_2$, present in many cell types, including pancreatic β cells ($cPLA_2\beta$), requires phosphorylation at Ser^{505} and binding with Ca^{2+} for its activity. Activation of $cPLA_2\beta$ would cause translocation of the enzyme to the secretory granules and accumulation of AA and lysophospholipids in the membrane, leading to changes in membrane structure or fluidity [2]. It has been demonstrated that $cPLA_2$ β plays a role in the maintenance of insulin stores [3] and its overexpression results in severe impairment of the calcium and secretory responses of β-cells to glucose [4].

The enzyme $iPLA_2\beta$ does not require Ca^{2+} for the catalytic activity and it is inhibited by the suicide substrate bromoenol lactone (BEL) [5]. It has been shown that $iPLA_2\beta$ inhibition prevents cell dysfunction associated with diabetes [6,7].$sPLA_2$ is contained in insulin secretory granules of pancreatic islet β-cells, it is co-secreted with insulin from glucose-stimulated islets [8] and it is expressed in human islets of transplanted pancreas after the recurrence of type 1 diabetes mellitus (T1DM) with insulitis [9,10].

The AA, released from membrane phospholipids by PLA_2 activities, has a significant regulatory and protective action on insulin secretion in pancreatic β cells [11]. These observations are consistent with $cPLA_2$ releasing AA in a controlled fashion from membrane phospholipids during insulin secretion. To date, studies examining the role of $cPLA_2$ and AA in the release of insulin have focused on short-term signaling. However, long-term exposure to high levels of fatty acids, including AA, results in desensitization and suppression of insulin secretion followed by induction of apoptosis [12].

It has been widely discussed at broad spectrum about the correlation between diabetes and the onset of bacterial infections [13,14] but, recently, particular attention has been paid to bacterial infections that may be responsible of the onset of diabetes by reduction of secretion of insulin by pancreatic cells [15,16]. Despite intensive research, a final conclusion concerning the causal role of microbes in the pathogenesis of T1DM has not been made.

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There are several proposed mechanisms of β-cell damage caused by microbes. It has been demonstrated that bacterial infection plays a significant role in bile-induced acute pancreatitis [17–19]. A case of acute cholecystitis and bacteremia with methicillin-resistant *Staphylococcus aureus* (MRSA) in a patient with HIV infection has been reported and highlights the invasive nature of staphylococcal infections [20]. Moreover, a study on obese mongrel dogs demonstrated that *Staphylococcus intermedius* infection leads to impaired insulin secretion [21]. *S. aureus* secretes numerous exotoxins, including a group of polypeptides capable of damaging the host cell plasma membrane. These polypeptides include pore-forming toxins (PFT: α-hemolysin, γ-hemolysin, δ-hemolysin, the Panton Valentine leukocidin, LukED, and LukGH/AB), β-hemolysin, and the phenol soluble modulins [22]. In particular, δ-hemolysin, a small amphipathic peptide with an α-helix structure could bind to the cell surface and aggregate to form transmembrane pores [23,24]. Our previous studies showed the significant role of $cPLA_2$, $iPLA_2$ and PKCα/ERK/MAPK signaling pathways during *E. coli* infection of microvascular endothelial cells [25,26]. The first aim of the present study was to investigate the role of PLA_2 and the involvement of PKCα/ERK1/2 signaling pathways in the response of the cells to *S. aureus* infection; the second was to demonstrate the effects of bacteria infection on insulin release.

2. Material and methods

2.1. Bacterial strains

S. aureus ATCC 33591 (methicillin-resistant strain) were grown in tryptic soy broth overnight at 37 °C. Bacteria were harvested by centrifugation for 10 min at 4300×*g* at 4 °C and washed twice in HBSS. The density of bacteria was measured by enumerating the number of CFU on LB agar plates (Difco).

2.2. Cell cultures

Rat insulinoma (INS-1E) β-cell line was kindly provided by Dr. C. B. Wollheim, (Médical Universitaire, Genève, Switzerland). INS-1E cells, widely used as a pancreatic β cell model [27,28], were cultured in RPMI-1640 medium containing as described [29].

2.3. Invasion assay

Cell monolayers (grown in 6-well tissue culture plate at a density of 8×10^5 cells/well) were infected with *S. aureus* (10^7 CFU/well) for 2 h, 4 h, 6 h and 8 h in a serum free medium. At the end of the incubation times, invasion was determined as described [25].

2.4. Electron microscopy

For transmission electron microscopy (TEM), cells were embedded in Durcupan ACM (Fluka Chemika-Biochemika). Ultrathin sections were double stained with uranyl acetate and lead citrate. Observations were carried out using a Hitachi H-7000 transmission electron microscope (Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.5. Insulin secretion assay

Glucose-induced insulin secretion was evaluated as previously described [29]. INS-1E cells (8×10^5 cells/well) were seeded in 6-well plates and incubated for 6 h in a serum free medium containing *S. aureus* (10^7 CFU/well) in presence or absence of 20 nM wortmannin plus 10 μM LY294002 (WTM/LY) or 20 μM PD98059 (kinase inhibitors) or 5 μM NS-398, COX-2 specific inhibitor.

At the end of the incubation period, gentamicin at 100 μg/ml was added and left for 1 h to kill extracellular bacteria. Cells were then washed three times with PBS and cultures were randomly divided into two groups. The first group (short-term infection) was stopped at this point (after incubation with *S. aureus* for 6 h) miming an acute infection. The second group (long-term infection), after 6 h of infection with *S. aureus*, was further incubated for another 72 h, in presence or absence of above mentioned inhibitors, in bacteria-free medium, containing 5 mM glucose in order to allow bacterial proliferation inside the cells and to mimic a chronic infection. Cells from the two groups were then incubated for 1 h at 37 °C in Krebs–Ringer-HEPES buffer (KRHB) [29] containing 2.7 mM glucose (starvation). Thereafter, cells were incubated for 2 h in the same buffer containing different concentrations of glucose (2.7 mM, 5.5 mM, 11.1 mM, 16.6 mM and 22.2 mM). Insulin levels in the culture media were measured by ELISA kit (Millipore).

2.6. Immunoblotting

The lysates of INS-1E cells were prepared for Western blotting as previously described [30–32]. Membranes were incubated with primary antibodies against total $cPLA_2$, $iPLA_2$, total PKCα, ERK 1/2, phospho-$cPLA_2$, phospho-PKCα, phospho-ERK 1/2, COX-1, COX-2 and α-actin, and then incubated with secondary antibodies for 1 h at room temperature.

2.7. Phospholipases A_2 activity assay

INS-1E cells were pre-incubated for 1 h in RPMI 1640 medium containing 5 mM glucose, supplemented or not with either 50 μM AACOCF3 or 2.5 μM BEL or 5 mM EDTA, or 20 nM wortmannin plus 10 μM LY294002 (WTM/LY) or 20 μM PD98059. The cells were then re-fed with fresh culture medium containing the inhibitors, in the presence or in the absence of *S. aureus* (10^7 CFU/well) for 6 h. Cells were then divided into two groups and processed as described in order to mime an acute and a chronic infection. Cells were lysed [31], and PLA_2s activity assays were performed by ELISA kit (Cayman Chemical Co.).

2.8. Transfection of siRNAs

The $cPLA_2$ and $iPLA_2$ knock-down in INS-1E cells was carried out by using rat ON-TARGET plus SMART pool *si*RNA duplex (Dharmacon), transfected by Lipofectamine RNAiMax (Life Technologies). Two sets of oligonucleotides were used: the first direct against $cPLA_2$ (Gene Bank NM_133551) and the second one direct against $iPLA_2$ (Gene Bank NM_001005560). After transfection with $iPLA_2$-*si*RNA or $cPLA_2$-*si*RNA, the cells were infected for 6 h with *S. aureus* (10^7 CFU/well). The cells were then divided in two groups, as described, and insulin release was determined.

2.9. Statistical analysis

Data is reported as mean ± standard deviation (SD). Statistical significance between two groups was analyzed by Student's *t*-test. One-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, was used to compare the means for the multiple groups. The P value <0.05 was considered statistically significant.

3. Results

3.1. Invasion of INS-1E by S. aureus

In Fig. 1, panel A, the percentage of invasion at 4 h and 6 h increased by 1.3- and 1.8-fold respectively in comparison with

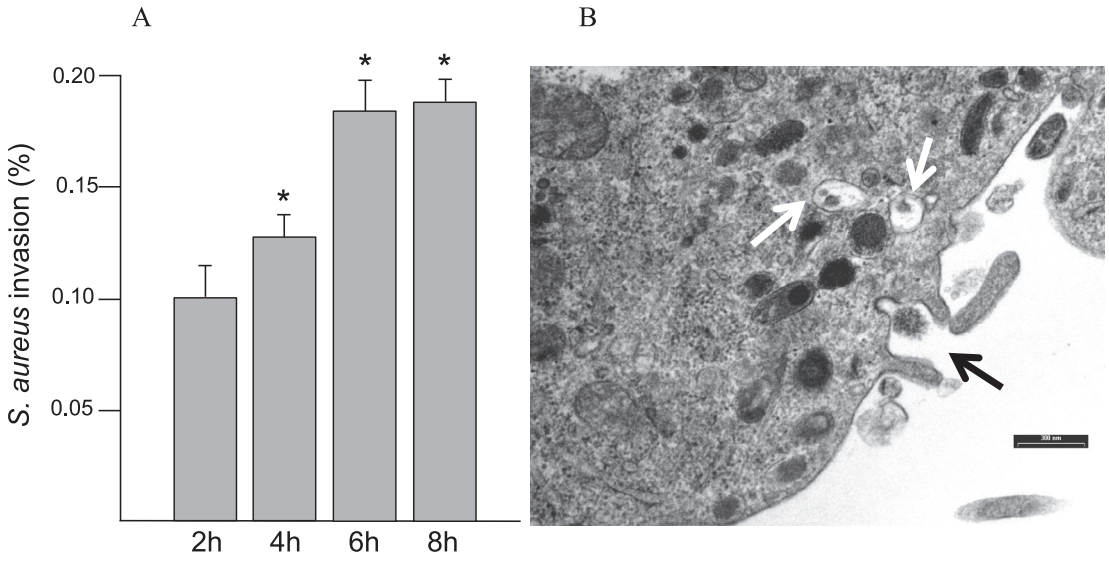

Fig. 1. Panel A: invasion assay after incubation of INS-1E cells with *S. aureus* for 2 h, 4 h, 6 h and 8 h. Values are expressed as a percentage of invasion ± SD by three independent experiments performed in triplicate. Statistically significant differences, by one-way ANOVA and the Tukey *post-test* are indicated ($^{*}P < 0.05$ *vs.* 2 h invasion). Panel B: Transmission Electron Microscopy (TEM) of INS-1E cells infected with *S. aureus* for 6 h. Bacteria are present, in contact with pseudopod-like structures on the surface of the cells and some bacteria are engulfed in the cytoplasm of the cells (white arrows). The bacteria appear to be in close contact with the cell membranes which encircle the microorganism (black arrow). Bar 300 nm.

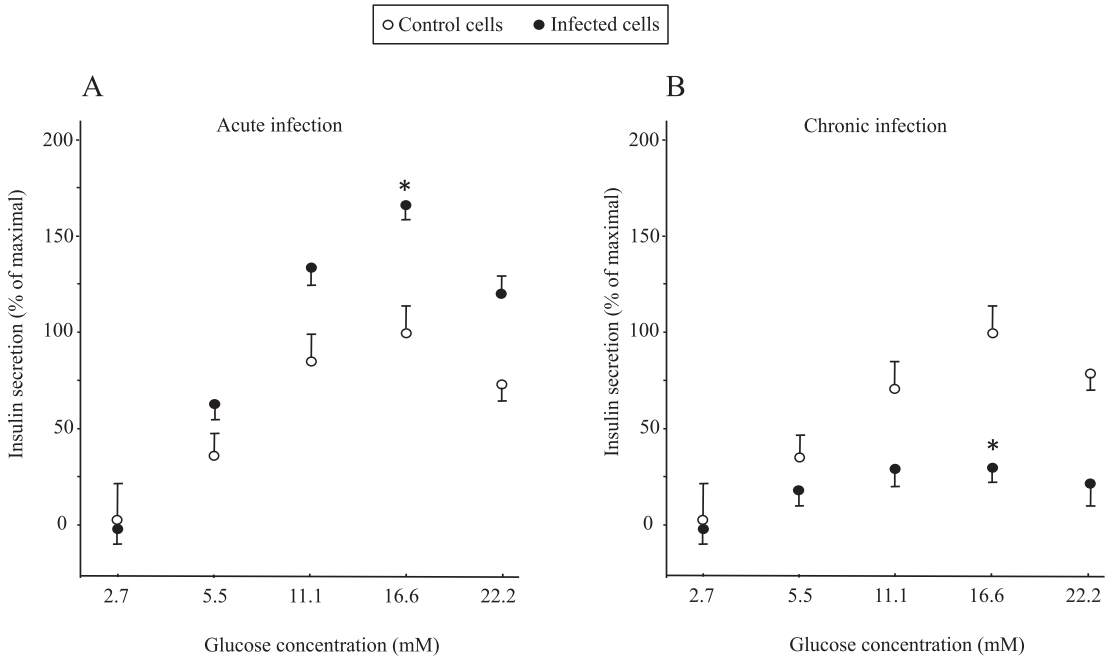

Fig. 2. Insulin release in INS-1E cells acute (panel A) or chronic (panel B) infection with *S. aureus*. Control cells were incubated in a medium without bacteria for 6 h (in acute *S. aureus* infection experiments) or for 6 h plus 72 h (in chronic infection experiments). In non-infected cells, the insulin release in presence of 16.6 mM glucose (maximal secretion) was 0.98 ± 0.07 ng/μg protein/h. Data is expressed as percentage of maximal secretion showed in INS-1E cells (mean ± SD measured by three independent experiments performed in triplicate), which for glucose stimulation is obtained at 16.6 mM. Statistically significant differences, by one-way ANOVA and the Tukey *post-test* are indicated ($^{*}P < 0.05$ vs. non-infected control cells at 16.6 mM glucose concentrations).

invasion after 2 h. In panel B, TEM images of INS-1E cells after infection for 6 h with *S. aureus* are shown. INS-1E cells formed microvilli-like protrusions that surrounded and endocytosed the bacteria, visible in the cytoplasm of the cells within vacuoles.

3.2. Insulin secretion

In Fig. 2, after acute infection, the insulin release significantly increased by 1.7-fold in presence of 16.6 mM glucose concentration in comparison to control cells (panel A). On the contrary, after chronic infection, insulin release in presence of 16.6 mM glucose concentration significantly decreased by 3.3-fold in comparison to non-infected cells at the same glucose concentration (panel B).

3.3. PLA_2, ERK 1/2, PKCα and COX-1/-2 expressions

In Fig. 3, panel A, after acute and chronic infection, no changes in the protein levels of total $cPLA_2$ in infected INS-1E in comparison to non-infected cells were observed. After acute infection,

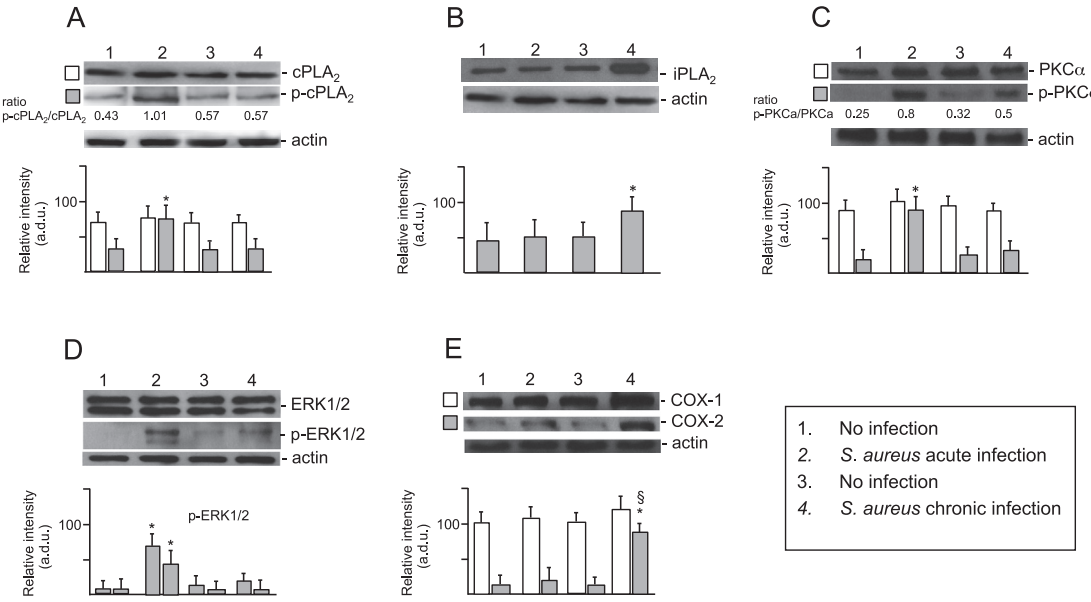

Fig. 3. Western blot analysis of $cPLA_2$ and phospho-$cPLA_2$ (A), $iPLA_2$ (B), PKCα and phospho-PKCα (C), ERK 1/2 and phospho-ERK 1/2 (D), and COX-1/2 (E) in INS-1E cells after acute and chronic infection with *S. aureus*. The values, expressed as arbitrary densitometric units (a.d.u.) were obtained by reading the blots using the ImageJ program and are the mean ± SD from three independent experiments ($n = 3$) performed in triplicate. GraphPad Prism was used to generate bar graphs. Statistically significant differences, determined by one-way ANOVA and the Tukey *post test*, are indicated ($P < 0.05$). (*) infected vs. non-infected cells at the same incubation period; (§) long-term infected vs. short-term infected cells (line 4 vs. line 2).

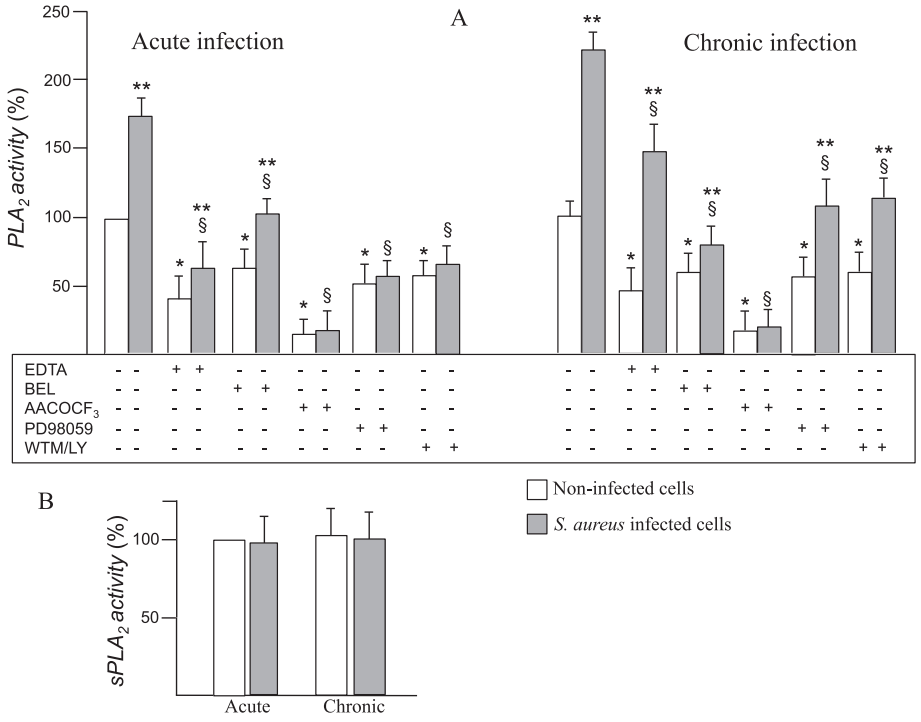

Fig. 4. Panel A: Phospholipase A_2 activities in INS-1E cells. $cPLA_2$ and $iPLA_2$ activities in non-infected cells and after acute and chronic infection (panel A) in absence or in presence of 50 μM AACOCF3 (both PLA_2s activity blocker) or 2.5 μM BEL ($iPLA_2$ inhibitor) or 5 mM EDTA ($cPLA_2$ inhibitor), or 20 nM WTM plus 10 μM LY294002 or 20 μM PD98059. The enzyme activity insensitive to BEL represents Ca^{2+}-dependent $cPLA_2$, whereas the enzyme insensitive to EDTA represents $iPLA_2$. Total PLA_2 specific activity was 18.8 ± 1.7 pmol/min/mg protein in control cells, 33.5 ± 2.9 pmol/min/mg protein after acute *S. aureus* infection and 47.6 ± 3.8 pmol/min/mg protein after chronic *S. aureus* infection in absence of inhibitors. Panel B: $sPLA_2$ activity in non-infected and in acute e chronic infected cells with *S. aureus*. Values, in percentage compared to control cells incubated in absence of bacteria (mean ± SD) are from three independent experiments ($n = 3$). Statistically significant differences, by one-way ANOVA and the Tukey *post-test* ($P < 0.05$) are indicated: (*) non-infected cells with inhibitors vs. non-infected w/o inhibitor cells; (§) infected cells with inhibitors vs. infected cells w/o inhibitor; (**) infected vs. non-infected in absence of inhibitor or in presence of the same inhibitor.

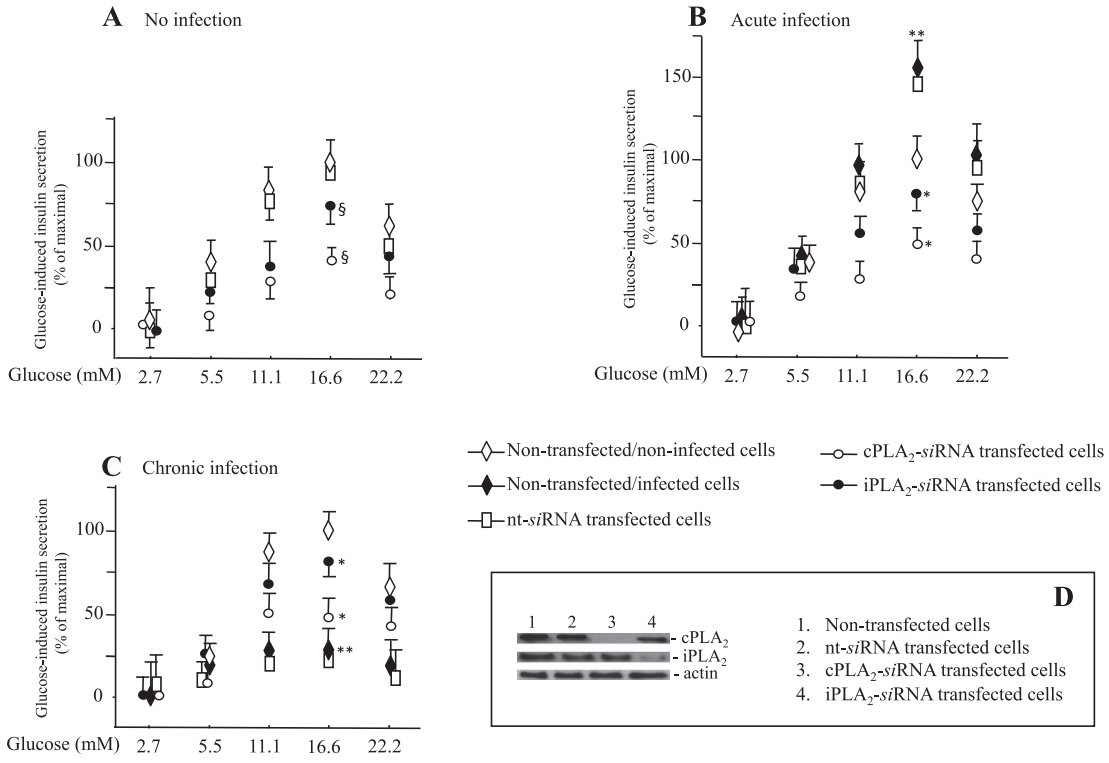

Fig. 5. Insulin release in INS-1E cells transfected with PLA_2-*si*RNAs. *si*RNAs used were provided as SMART pool designed against shared and conserved regions in order to ensure efficient and specific target silencing for cPLA$_2$ α, β and γ, as well as iPLA$_2$, as indicated by the provider. A *si*RNA non-targeting was used as negative control. (A) insulin secretion of cPLA$_2$- and iPLA$_2$-*si*RNA transfected cells without *S. aureus* infection. (B) insulin secretion of cPLA$_2$- and iPLA$_2$-*si*RNA transfected cells after acute *S. aureus* infection. (C) insulin secretion of cPLA$_2$- and iPLA$_2$-*si*RNA transfected cells after chronic *S. aureus* infection. (D) cell lysates were immunoblotted to confirm the reduction of PLA_2 protein levels. Data is expressed as percentage of maximal secretion showed in non-transfected/non-infected INS-1E cells (mean ± SD measured by three independent experiments performed in triplicate), which for glucose stimulation is obtained at 16.6 mM glucose concentration. Statistically significant differences, by one-way ANOVA and the Tukey *post-test* ($P < 0.05$) are indicated at 16.6 mM glucose concentration: (§) transfected cells vs. non-transfected/non-infected cells (in panel A); (*) transfected cells vs. non-transfected/infected cells; (**) non-transfected/infected cells (in panels C and D) vs. non-infected cells.

phospho-cPLA$_2$ significantly increased (1.01 ratio p-cPLA$_2$/cPLA$_2$). Calcium-independent PLA_2 expression (panel B) increased by about 1.8-fold in *S. aureus* infected INS-1E cells after chronic infection in comparison to control cells at the same incubation period.

Since PKCα and p42/p44 MAPK might be involved in stimulated cPLA$_2$ activity, PKCα and ERK 1/2 protein expressions were evaluated. After acute infection, phospho-PKCα and phospho-ERK 1/2 expressions significantly increased in comparison to non-infected cells at the same incubation period (panels C and D). The very low levels of both phospho-PKCα and phospho-ERK 1/2 expression after chronic infection might be explained by the fact that levels of phosphorylated protein, with low half-life, are drastically reduced after 72 h of incubation with bacteria.

Furthermore, COX-2 expression (panel E) significantly increased after chronic infection by 4.1-fold in comparison to the respective control non-infected cells at the same incubation period, and by 3.2-fold in comparison to acute-infected cells. No changes after acute infection and a weak increase (1.2-fold) after chronic infection, in COX-1 expression were observed.

3.4. PLA_2 activities

In Fig. 4, PLA_2 activity in INS-1E cells after acute or chronic infection with *S. aureus* in absence or in presence of PLA_2 inhibitors are shown.

PLA_2 activity of non-infected INS-1E cells (panel A) decreased by almost 2.5 and 1.5-fold in presence of EDTA and BEL, respectively, in comparison to control cells in absence of inhibitors. Specific activity was reduced to a very low level by AACOCF3 (cPLA$_2$ and iPLA$_2$ activity inhibitor). PD98059 and WTM/LY reduced PLA_2 activity of non-infected cells by about 1.8 and 1.6-fold, respectively, in comparison to control cells in absence of inhibitors. After acute infection, PLA_2 activity was significantly activated (by almost 1.7-fold) compared with non-infected control cells. The infection in presence of EDTA, BEL or AACOCF3 caused a significant decrease of PLA_2 activity by 2.6, 1.7 and 8.5-fold, respectively, in comparison to infected cells in absence of inhibitors. The significant decrease of enzyme activity in presence of EDTA suggests a greater contribution of cPLA$_2$ in mediating AA release following *S. aureus* acute infection.

After chronic infection, PLA_2 activity of INS-1E cells was significantly activated (by almost 2.2-fold) compared to non-infected control cells. The incubation of INS-1E cells with *S. aureus* in presence of EDTA caused a decrease of PLA_2 activity by 1.4-fold in comparison to infected cells in absence of inhibitor. Surprisingly, BEL decreased PLA_2 activity by 2.5-fold, highlighting that, following *S. aureus* chronic infection, iPLA$_2$ activity is mainly responsible for the AA production. After chronic infection in presence of PD98059 and WTM/LY, PLA_2 activity significantly decreased by 2.0 and 1.8-fold, respectively, in comparison to infected cells in absence of inhibitors. No differences in sPLA$_2$ activity were found after short- or long-term infection (panel B), indicating that sPLA$_2$ is not involved in the response of the cells after *S. aureus* infection.

Bacterial PLA_2 was also assayed, but its contribution in the bacterial concentration used in our experiments was undetectable.

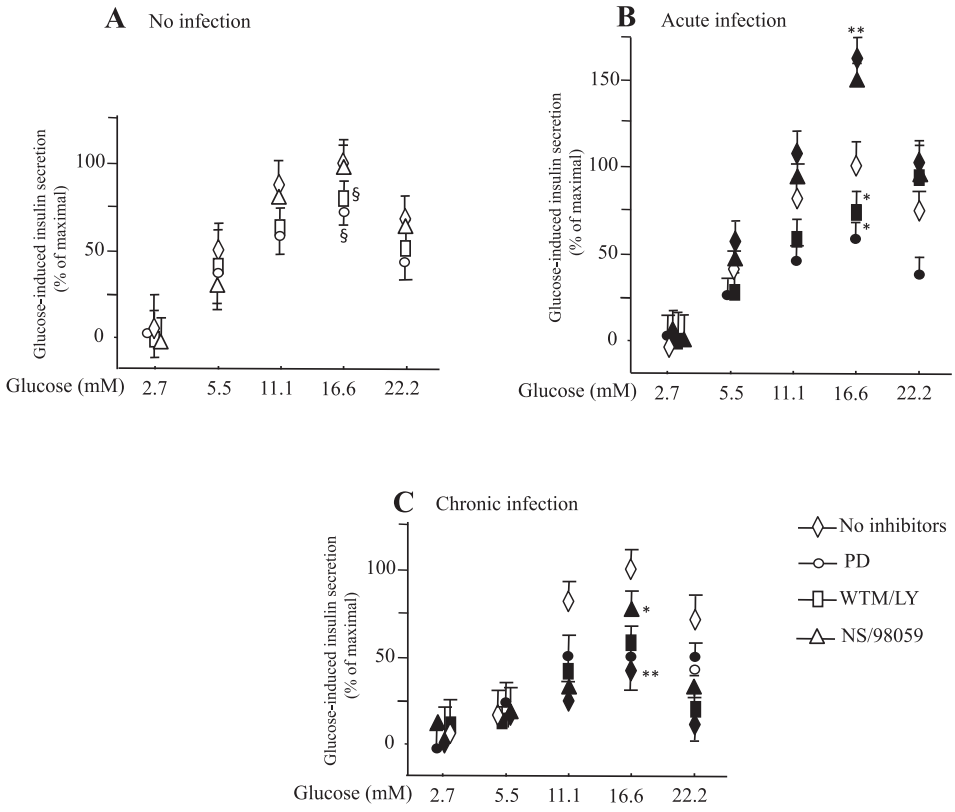

Fig. 6. Insulin release in INS-1E cells in presence or absence of inhibitors. None of these components, used at the specified concentration, affected cell viability, as verified by trypan blue exclusion test (data not shown). (A) insulin secretion of INS-1E cells without *S. aureus* infection. (B) insulin secretion of cells after acute *S. aureus* infection. (C) insulin secretion of cells after chronic *S. aureus* infection. INS-1E cells were pre-incubated for 1 h with 20 nM WTM plus 10 μM LY294002 or 20 μM PD98059 or 5 μM NS-398. Infection was performed in presence of these inhibitors. Data is expressed as percentage of maximal secretion showed in non-transfected/non-infected INS-1E cells (mean ± SD measured by three independent experiments performed in triplicate), which for glucose stimulation is obtained at 16.6 mM glucose concentration. Statistically significant differences, by one-way ANOVA and the Tukey *post-test* ($P < 0.05$) are indicated at 16.6 mM glucose concentration:empty symbols indicate results in absence of *S. aureus* infection and filled symbols indicate infected cells (acute or chronic infection); (§) non-infected cells with inhibitors vs. non-infected w/o inhibitor cells (in panel A); (*) infected cells with inhibitors vs. infected cells w/o inhibitor; (**) non-transfected/infected cells (in panels B and C) vs. non-infected cells.

3.5. Transfection of $cPLA_2$- and $iPLA_2$-siRNA

In Fig. 5, in non-infected cells (panel A), the insulin release at 16.6 mM glucose concentration significantly decreased by 1.3 and by 2.2-fold in $iPLA_2$- and $cPLA_2$-*si*RNA transfected cells, respectively, in comparison to non-transfected INS-1E cells. After acute infection (panel B), insulin release at 16.6 mM increased by 1.6-fold in comparison to non-transfected/non-infected cells at the same glucose concentration. Moreover, insulin release significantly decreased by 2.0 and by 3.2-fold in $iPLA_2$- and $cPLA_2$-*si*RNA transfected cells, respectively, in comparison to non-transfected INS-1E cells. After chronic infection (panel C), insulin release at 16.6 mM decreased by 3.1-fold in comparison to non-transfected/non-infected cells at the same glucose concentration. In these conditions, insulin release by $iPLA_2$- and $cPLA_2$-*si*RNA transfected INS-1 cells at 16.6 mM glucose concentrations increased by 2.6 and by 1.5-fold respectively, in comparison to non-transfected/chronic-in fected cells.

In panel D, immunoblots of INS-1E lysates after transfection revealed the specificity of *si*RNAs used: both PLA_2 protein basal expressions were strongly attenuated in transfected unstimulated cells, by approximately 95% for $cPLA_2$ and 85% for $iPLA_2$, compared to the levels in non-transfected cells or transfected with non-targeting *si*RNA.

3.6. Insulin secretion in presence of inhibitors

In Fig. 6, in non-infected cells (panel A), the insulin release at 16.6 mM glucose concentration decreased by 1.2 and by 1.4-fold after incubation with WTM/LY and PD98059, respectively, in comparison to INS-1E cells in absence of inhibitors. No change in the insulin secretion after incubation with NS-398 was found. After acute infection, the insulin release significantly increased by 1.6-fold in presence of 16.6 mM glucose concentration in comparison to control cells (panel B). After incubation with WTM/LY and PD98059, insulin release decreased in acute-infected cells by 2.1 and 2.6-fold, respectively. After chronic infection (panel C), insulin release significantly decreased by 2.2-fold in presence of 16.6 mM glucose concentration in comparison to non-infected cells. Incubation with NS-398 significantly increased the insulin secretion by about 1.6-fold in comparison to chronic-infected cells in absence of inhibitors. Weak changes in the insulin release after incubation of chronic-infected cells with WTM/LY and PD98059 in comparison to chronic-infected cells in absence of inhibitors were found.

4. Discussion

Participation of PLA_2s in glucose-induced insulin secretion is reflected by the fact that secretion is impaired by suppressing their expression or by their pharmacologic inhibition [33,34]. Bacterial infection can lead to the impairment in insulin secretion [21] but the system(s) responsible of this effect has not been established. Attractive candidates are the PLA_2s because their activity during insulin release by β cells.

Here we have investigated the effects of bacterial infection by using two experimental models, one miming an acute infection and the other miming a chronic infection by *S. aureus* of INS-1E cells, able to secrete insulin in response to elevated glucose

concentrations. The glucose concentration-dependence curve for these cells is similar to that of rat islets and, for this reason, INS-1E cells represent a stable and valuable β cell model [27,28,35].

It has been demonstrated that bacterial infection may reduce or increase the secretion of insulin based on the type of microorganism which penetrate the pancreatic tissue [15]. *S. aureus* is a Gram-positive bacterium that causes both community- and hospital-acquired infections [36]. Experimental studies have shown that the translocation of bacteria in the blood, and therefore in the pancreas, plays a significant role in the pathogenesis of pancreatitis [37]. Moreover, it has been demonstrated that long-term exposure to high levels of fatty acids is detrimental to β cell function [38] in contrast to the stimulatory short-term effects of exogenous AA and other fatty acids on the β cells [39,40].

The results obtained in this study show that, after acute *S. aureus* infection, insulin secretion by the *S. aureus*-infected cells is higher compared to uninfected ones. After chronic infection, insulin secretion is significantly reduced and at the same time extremely high PLA_2 activities and expressions were observed. We speculated that the high AA concentration, could cause a dangerous imbalance through the up-regulation of COX-2 enzymes which release a high amount of prostaglandins. Our results demonstrated that COX-2 expression is higher after chronic infection in comparison to protein expression in non-infected cells and that the inhibition with NS-398 induces a restore of insulin secretion in *S. aureus* treated cells (chronic infection), indicating that the products of COX-1/2 play a relevant role by inhibiting insulin release. In diabetic islet, it has been demonstrated that increased PGE_2 secretion, mediates a signaling pathway that negatively regulates insulin secretion, contributing in the β cell dysfunction [41].

Moreover, after acute infection, PKCα and ERK 1/2 activation by phosphorylation was evident, demonstrating their involvement during *S. aureus* infection and providing new insight in understanding molecular mechanisms through which bacterial-induced impairment insulin secretion occurs. PKCα phosphorylates a number of proteins involved in various signal transduction processes, including $cPLA_2$ [42]. These results were confirmed by the reduction of $cPLA_2$ and $iPLA_2$ activity after incubation of INS-1E cells with *S. aureus* in presence of PD98059 or WTM/LY294002 and by the reduction of insulin release after acute infection of the cells in presence of PD98059 or WTM/LY294002 compared to infected cells without inhibitors.

Interestingly, insulin secretion in *si*RNA-$iPLA_2$- is higher than in *si*RNA-$cPLA_2$-transfected cells, highlighting that $iPLA_2$ plays a main role in insulin secretion after chronic infection of INS-1E cells. Thus, $iPLA_2$ could represent a therapeutic target to moderate the AA concentration imbalance, presumed responsible for the insulin secretion reduction in *S. aureus* infected β cells.

Pore formation, after *S. aureus* infection, leads to a rapid rise in intracellular calcium concentration associated with either direct influx through the pore or with a release from intracellular compartments [23]. An increase in $[Ca^{2+}]i$ could lead to relocation of $cPLA_2$ to the phospholipid-containing plasma membrane and trigger its phosphorylation through a calmodulin-dependent kinase, leading to greater catalytic activity [24]. We speculated that the phosphorylation of PKCα and ERK 1/2 after bacterial infection could play a role also in $iPLA_2$ activation by tyrosine phosphorylation [43,44].

The results of this study reveals a novel pathway whereby *S. aureus* chronic infection impairs insulin secretion by INS-1E cells, thus elevating PKCα and ERK 1/2 activities which in turn activate PLA_2s and modulate the insulin release after *S. aureus* infection. Moreover, we demonstrated that $iPLA_2$ play a main role in the response to the chronic *S. aureus* infection of INS-1E cells.

Further studies on ability of the bacteria to modulate insulin secretion of other cell lines or isolated islet are needed to understand the mechanism through which they could cause diabetes, for the development of strategies for the prevention of insulin imbalance and for the implementation of new therapeutic approaches.

Conflict of interest

The authors declare no conflict of interest.

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