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Low-protein diet disrupts the crosstalk between the PKA and PKC signaling pathways in isolated pancreatic islets

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Abstract

Protein restriction in the early stages of life can result in several changes in pancreatic function. These alterations include documented reductions in insulin secretion and in cytoplasmic calcium concentration $[Ca^{2+}]_i$. However, the mechanisms underlying these changes have not been completely elucidated and may result, in part, from alterations in signaling pathways that potentiate insulin secretion in the presence of glucose. Our findings suggest that protein restriction disrupts the insulin secretory synergism between Cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) and Ca^{2+}-dependent protein kinase C (PKC) in isolated islets. Western blot analysis demonstrated reduced levels of both phospho-cAMP response element-binding protein (phospho-CREB) at Ser-133 and substrates phosphorylated by PKCs (Phospho-(Ser) PKC substrate), suggesting that PKA and PKC activity was impaired in islets from rats fed a low-protein diet (LP). cAMP levels and global Ca^{2+} entry were also reduced in LP islets. In summary, our findings showed that protein restriction altered the crosstalk between PKA and PKC signaling pathways, resulting in the alteration of secretory synergism in isolated islets.

Keywords: Low-protein diet; Crosstalk; PKA; PKC; Insulin secretion

1. Introduction

A relationship between malnutrition and chronic diseases has been observed in populations in developing countries [1]. Severe malnutrition in children is related to a disturbance in beta cells [2], a reduction in pancreatic beta-cell mass and a lower responsiveness to glucose by the remaining insulin-producing cells [3–6]. In addition, alterations in enzymes involved in the secretory process have been found in islets from malnourished animals, leading to reductions in intracellular Ca^{2+} oscillations and insulin secretion [7–9].

Although Ca^{2+} influx is essential for the process of exocytosis, the absolute magnitude of the secretory response is highly dependent on the phosphorylation of key elements involved in the exocytotic process [10–12]. However, this stimulus is weak compared with the large exocytotic responses that are evoked by the same amount of Ca^{2+} entry when PKA and/or PKC are activated [11]. In addition, the activation of PKA in pancreatic beta cells can recruit and direct insulin granules toward exocytosis [13]. Furthermore, rats that are genetically modified to have increased expression of the catalytic subunit of PKA secrete more insulin [14].

Several lines of evidence demonstrate that PKA is required for the stimulation of transcription by cAMP [15]. Thus, PKA links cAMP formation to gene transcription by phosphorylating nuclear cAMP response element binding protein (CREB) at Ser-133 [16], suggesting that CREB is indeed a bona fide substrate for this kinase [17] and a key transcription factor in the maintenance of efficient glucose sensing, insulin exocytosis, insulin gene transcription and beta-cell survival [16,18–21].

Several studies have shown that concomitant administration of stimuli that activate the PKA and PKC pathways resulted in an additive or synergistic increase in CREB-mediated transcription in other tissues [22,23], suggesting that the PKC phosphorylation of CREB regulates its dimerization [24]. Furthermore, PKC activation is associated with the

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phosphorylation and remodeling of actin microfilaments near the plasma membrane, an event that facilitates the docking and exocytosis of vesicles containing insulin granules in beta cells [25,26].

The simultaneous activation of PKA and PKC with forskolin (FSK) and phorbol 12-myristate 13-acetate (PMA) enhances the secretion of insulin in the presence of glucose more efficiently than when the kinases are activated separately [27]. PKA and PKC in conjunction promote extensive exocytosis [10]. Moreover, there are reports of crosstalk between PKA and PKC cascades in other tissues [28,29]. In nervous system tissues, PKC increases the activity of adenylyl cyclases (AC) and the level of cAMP [30]. In addition, PKC activation are involved in the modulation of AC activity in pancreatic islets [31].

In beta cells, specific isoforms of AC and phosphodiesterase can effectively influence and synchronize cAMP dynamics [32], which then allows cAMP to facilitate the action of Ca^{2+} by stimulating secretory granule exocytosis [33]. It has been previously reported that the elevation of intracellular cAMP levels promotes Ca^{2+} mobilization from intracellular stores by activating Ca^{2+}-induced Ca^{2+} release [34,35]. These results suggest that cAMP facilitates Ca^{2+} influx in beta cells by modulating the gating properties of the calcium channels [36]. Moreover, there is accumulating evidence that cAMP, via activation of PKA, accelerates granule mobilization in beta cells [37].

The L-type voltage-gated Ca^{2+} channels mediate a substantial fraction of insulin secretion from pancreatic beta cells and are the primary pathway to promote an increase in cytosolic $[Ca^{2+}]_i$ [38]. Moreover, the α-subunit of the voltage-dependent calcium channels (VDCCs) can be phosphorylated by PKA, which increases the probability of the channel opening and increases the $[Ca^{2+}]_i$ levels in beta-cell lines [39,40]. By contrast, islets from rats fed a low-protein diet showed decreased cytosolic $[Ca^{2+}]_i$ levels [41,42], and the calcium current was reduced by diminished PKC activation in beta cells, suggested that PKC may regulate Ca^{2+} influx by changing the phosphorylation state of the L-type VDCC [43]. However, to date, there has been no direct demonstration of PKC-mediated phosphorylation of beta cell VDCC subunit(s) [44].

Glucose can stimulate insulin secretion in a Ca^{2+}-independent manner during muscarinic and incretin stimulation [45]. Reports on the secretory synergism between PKC and PKA in beta cells are scarce [27,46,47]. Although protein kinases have been implicated in the control of insulin secretion, precisely how they participate in producing a controlled insulin response is not well understood. In the present study, we found that protein restriction disrupted the synergistic crosstalk between the PKA and PKC pathways during the insulin secretory process.

2. Materials and methods

2.1. Ethical aspects

All of the procedures used in this study are in accordance with the standards suggested by the Brazilian College of Animal Experimentation and with the international guidelines established by the National Institute of Health Guide for Care and Use of Laboratory Animals. The research was approved by the Ethics Committee on Animal Use of the Federal University of Pernambuco, protocol number 23076.041290/2012-13.

2.2. Animals and diet

Groups of 5 male Wistar rats (21 days old) from the breeding colony at the Central Animal Facility of the State University of Campinas (UNICAMP) São Paulo – Brazil, were kept at 22±2°C with a 12-h light:dark cycle. The rats were randomly assigned to groups that were fed an isocaloric diet containing 6% (low-protein diet, LP) or 17% (normal protein diet, NP) protein for 8 weeks (Table 1), as described earlier [48]. At the end of the experimental period, the nutritional status of the rats was evaluated by measuring body weight. Biochemical parameters were measured at the end of the experiment for establishment of efficiency and characterization of the model. Before decapitation, glucose was measured in blood obtained from the tail tip (Accu Chek Advantage II glucometer). On the day after the 8-week treatment, fasted rats were euthanized by CO_2 exposure, followed by decapitation. The trunk blood was collected and centrifuged, and the serum was used for the measurement of the following parameters in overnight fasted rats: protein and albumin (In Vitro Diagnostica, Itabira, Brazil) and nonesterified fatty acids (NEFA) (non-esterified fatty acid C kit, Wako Chemicals, Richmond, VA, USA) according to the manufacturer`s instructions. Liver glycogen and fat levels were determined as previously reported [49,50].

Table 1
Composition of the normal and low protein diets [1]

Ingredient	Normal protein (17%) protein	Low protein (6% protein)
Casein (84%) protein	202	71.5
Cornstarch	397	480
Dextrinized cornstarch	130.5	159
Sucrose	100	121
Soybean oil	70	70
Fiber	50	50
Mineral mix	35	35
Vitamin mix	10	10
L-Cystine	3	1
Choline chlorhydrate	2.5	2.5

[1] See Ref. [48] for more details.

2.3. Islet isolation and insulin secretion

The pancreatic islets were isolated with the collagenase method, as previously described [51]. For static incubation, groups of five size-matched islets were first incubated for 45 min at 37°C in Krebs-bicarbonate buffer with the following composition: 115 mM NaCl, 5 mM KCl, 2.56 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM $NaHCO_3$, 15 mM HEPES and 5.6 mM glucose. The buffer was supplemented with 3 g of bovine serum albumin/L and equilibrated with a mixture of 95% O_2:5% CO_2, pH 7.4. This medium was then replaced with fresh buffer, and the islets were incubated for 1 h with 2.8 or 8.3 mM glucose/L and FSK (10 μM) and/or PMA (200 nM) as indicated. The insulin content of the medium at the end of the incubation period was measured by RIA [52].

2.4. Western blot

Isolated islets from NP and LP rats were incubated in Krebs solution supplemented with 0.5% bovine albumin (m/v), FSK (10 μM) and PMA (200 nM) in presence of glucose (8.3 mM) for 4 h. Subsequently, the islets were solubilized in homogenization buffer containing the following: 100 mM Tris pH 7.5, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF and 1% Triton X-100. The islets were disrupted using a sonicator (Brinkmann Instruments, Westbury, NY, USA), employing three 10-s pulses. The extracts were then centrifuged at 12,600×g at 4°C for 5 min to remove insoluble material. The protein concentration in the supernatants was assayed using the Bradford dye method [53]. For sodium dodecyl sulfate gel electrophoresis and Western blot analysis, the samples were treated with Laemmli sample buffer containing dithiothreitol. After heating to 95°C for 5 min, the proteins were separated by electrophoresis (40 μg protein/lane, 10% gels). Following electrophoresis, proteins were transferred to nitrocellulose membranes and treated overnight with blocking buffer (5% non-fat dried milk, 10 mM Tris, 150 mM NaCl and 0.02% Tween-20, final pH 7.4) and were subsequently incubated with phospho-(Ser) PKC substrate antibody (1:1000, cat #2261, Cell Signaling), phospho-CREB (Ser-133) antibody (1:1000, cat #9196, Cell Signaling) and GAPDH (1:1000, sc-25778, Santa Cruz) used as an internal control. Proteins were detected using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL) after a 2-h incubation with a horseradish peroxidase-conjugated secondary antibody (1:10,000, Invitrogen, São Paulo, SP, Brazil). Band intensity was quantified using the free software ImageJ Tool (http://ddsdx.uthscsa.edu/dig/itdesc.html).

2.5. Measurement of intracellular cyclic AMP content

The cAMP content of islets was determined in the presence of FSK with the cAMP Enzyme immunoassay system (Amersham cAMP Biotrak Enzyme Immunoassay System #RPN225; Amersham Life Sciences) according to the manufacturer's instructions.

2.6. Measurement of cytoplasmic Ca^{2+}

Islets were pre-incubated in RPMI 1640 medium containing 5.6 mM glucose and 10% fetal bovine serum (FBS) and kept at 37°C for 2 h. For the final hour of pre-incubation, islets were loaded with 5 μM Fura-2 acetoxymethylester (AM), a calcium-sensitive dye. Afterwards, single islets were placed inside a thermostatically regulated chamber (37°C) over polylysine-treated glass coverslips and perifused with albumin-free Krebs buffer containing 2.8 or 8.3 mM glucose in association with 10 μM FSK or 200 nM PMA, as indicated. Cells loaded with Fura-2 were imaged with an inverted epifluorescence microscope (Nikon Eclipse TE200, Tokyo, Japan). A ratio image was acquired approximately every 3 s with a Cool One camera (Photon Technology International, NJ, USA) with a dual filter wheel equipped with 340 nm, 380 nm and

10 nm bandpass filters and a range of neutral density filters (Photon Technology International, NJ, USA). Data were acquired with Image Master software, version 5.0 (Photon Technology International).

2.7. Statistical analysis

Values shown are means±S.E.M. Student's unpaired *t* test was used to compare the body weight, protein, albumin, glucose, NEFA, and insulin, as well as the liver glycogen, fat and cAMP levels. To compare the changes in insulin secretion, calcium influx and western blot results, the data were log-transformed to correct for heterogeneity in variance and then analyzed by two-way ANOVA, followed by the Tukey-Kramer test to determine significant differences between groups and among glucose and secretagogue concentrations, as well as to assess the interactions between these factors. The data were analyzed using a statistical software package (Statsoft, Tulsa, OK). The level of significance was set at $P<.05$.

3. Results

The LP diet treatment successfully induced malnutrition, as indicated by the rats' lower body weight, serum proteins and albumin compared with the NP rats ($P<.05$; Table 2). The serum insulin in the postprandial period was lower in LP vs. NP rats ($P<.05$). There were no significant differences between either the fasting serum glucose or the fasting serum insulin in LP vs. NP rats. Fasting non-esterified fatty acids (NEFA), liver fat and glycogen were all higher in the LP group compared with the NP group ($P<0.05$; Table 2).

Fig. 1 shows the effect of FSK and/or PMA on insulin secretion in both groups of islets incubated for 1 h in the presence of 2.8 or 8.3 mM glucose. In the presence of 2.8 mM glucose, there was no significant difference between the groups. However, at 8.3 mM glucose, the LP group showed reduced insulin secretion compared with NP islets ($P<.05$). The individual addition of FSK or PMA raised insulin secretion in both islet groups ($P<.05$). However, the increase in insulin secretion was significantly lower in LP vs. NP islets ($P<.05$). Furthermore, the simultaneous addition of FSK and PMA significantly increased insulin secretion only in the NP group ($P<.05$); the simultaneous stimulation of LP islets with FSK and PMA promoted no further increase in insulin secretion.

Next, we assessed the level of phospho-CREB (Ser-133) in isolated islets after a 4 h incubation with 10 μM FSK (Fig. 2A) or 200 nM PMA (Fig. 2B) in the presence of 8.3 of glucose. At basal conditions, LP islets showed lower CREB phosphorylation than NP ($P<.05$). Incubation with FSK increased phospho-CREB levels in both groups ($P<.05$); however, this increase in CREB phosphorylation was significantly lower in the LP islet group ($P<.05$). Incubation with PMA also increased phospho-CREB levels in both groups ($P<.05$), although also to a lesser extent in LP islets ($P<.05$).

In the following experiments, activation of the PKC pathway was assessed using antibodies against specific motifs phosphorylated by PKC after a 4 h incubation with 200 nM PMA (Fig. 3A) and 10 μM FSK (Fig. 3B) in the presence of 8.3 mM of glucose. Under basal conditions, LP islets showed lower PKC-induced substrate phosphorylation ($P<.05$; Fig. 3A and B). Incubation with PMA led to an increase in PKC-induced phosphorylation in both groups ($P<.05$; Fig. 3A), but to a lesser extent in LP islets ($P<.05$). Moreover, neither of the islet groups showed PKC-dependent phosphorylation events after incubation with FSK (Fig. 3B).

Table 2
The effect of protein restriction on rats fed a normal protein (NP) or low-protein (LP) diet for 8 weeks; values are the means±S.E.M.

	Groups					
	NP			LP		
Parameters	*n*	Mean	S.E.M.	*n*	Mean	S.E.M.
Body weight [1], g	10	265.0	10.0	10	215.0 *	15.0
Protein [1], g/dl	10	4.9	0.2	10	4.0 *	0.3
Albumin [1], g/dl	10	3.5	0.1	10	3.1 *	0.1
Glucose [1], mg/dl	10	129.4	74.0	10	132.4	14.6
NEFA [1], mM	10	0.4	0.01	10	0.7 *	0.09
Insulin [1], ng/ml	10	0.98	0.13	10	0.85	0.18
Insulin [2], ng/ml	10	2.25	0.6	10	0.95 *	0.2
Liver glycogen [1], mg/100 mg tissue	10	0.7	0.1	10	1.40 *	0.1
Liver fat [1], g FA/100 g tissue	10	6.8	0.5	10	12.4 *	0.9

* Different from NP rats, $P<.05$, according to Student's *t* test for independent samples.
[1] These values were obtained from fasting rats.
[2] These values were obtained from fed rats.

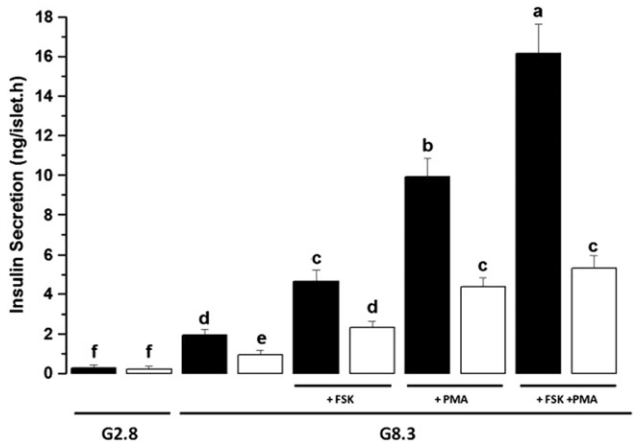

Fig. 1. Forskolin (10 μM) and PMA (200 nM)-induced insulin secretion in NP and LP islets. The columns represent the cumulative 1 h insulin secretion and are the means± S.E.M.; $n=15$ from 3 independent experiments. Different letters indicate a significant difference ($P<.05$) according to 2-way ANOVA.

In the next series of experiments, we analyzed Ca^{2+} oscillation dynamics in a single isolated islet perifused with basal (2.8 mM) or stimulatory (8.3 mM) glucose in combination with 10 μM FSK (Fig. 4A) or 200 nM PMA (Fig. 5A). Perifusion with 8.3 mM of glucose increased the transient rise in Ca^{2+} and raised the global Ca^{2+} entry as measured by the increased area under the curve (AUC) in both groups, although the increase was significantly lower in the LP group (Figs. 4B and 5B). The addition of PMA to the perifusion system increased the transient rise in Ca^{2+} (Fig. 5A) and global Ca^{2+} entry ($P<.05$; Fig. 5B) in both groups, although the increase was higher in NP islets. The addition of 8.3 mM of glucose plus FSK to the perifusion system led to increased Ca^{2+} oscillations in both groups (Fig. 4A); surprisingly, the transient rise in Ca^{2+} was higher in LP islets. However, the global Ca^{2+} entry, represented by the AUC, was significantly lower in the LP group compared with the NP group in the presence of FSK ($P<.05$; Fig. 4B). Ultimately, intracellular cAMP levels were significantly reduced in LP islets compared with islets in the NP group in response to 10 μM FSK ($P<.05$; Fig. 6).

4. Discussion

In our study, rats fed a LP diet showed lower body weight and similar biochemical parameters as in several other studies [7,8,54,55], demonstrating that these rats were a suitable model. Although insulinemia was reduced, glycemia was unchanged in rats fed either a LP or NP diet. This finding may be due in part to a marked increase in insulin sensitivity resulting from the increased phosphorylation of the insulin receptor and insulin receptor substrate-1 as well as its association with phosphatidylinositol 3-kinase in muscle and liver tissues [3,55–59]. Regarding albumin levels, humans and rats with genetic alterations that result in decreased levels of albumin show increased levels of plasma lipids and lipoproteins [60–63]. Moreover, the increase in NEFA may be related to a reduction in glucose-induced insulin secretion [64,65]. These events may partially explain the increase of NEFA and the reduction of insulin secretion in the presence of glucose in our experimental model. Consistent with several studies

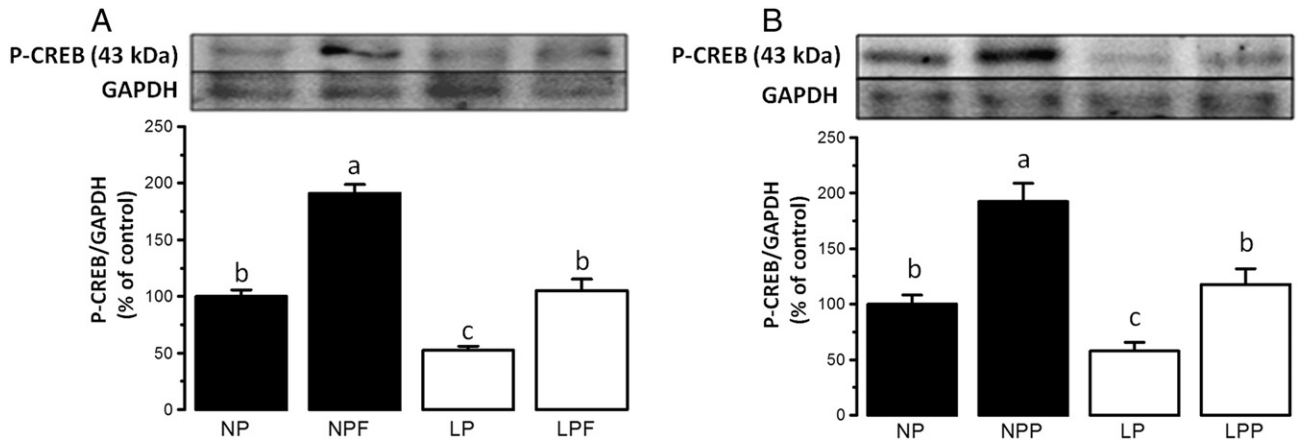

Fig. 2. A and B. Western blot analysis of phospho-CREB (Ser-133). Isolated islets from NP and LP rats were incubated in Krebs solution supplemented with 0.5% bovine albumin (m/v), FSK (10 μM) and PMA (200 nM) in the presence of 8.3 mM glucose for 4 hours. The columns are the means±S.E.M., n=5, from 2 independent experiments. Different letters indicate a significant difference (P<.05) according to 2-way ANOVA. NPF=NP +10 μM FSK; LPF=LP+10 μM FSK; NPP=NP+200 nM PMA; LPP=LP+200 nM PMA.

[7,8,54,66], LP islets showed a higher level of hepatic glycogen compared with NP islets (P<.05). The increase in hepatic glycogen may be explained by the up-regulation of insulin signaling and subsequent inhibition of gluconeogenesis in the rat liver [59].

Because FSK and PMA strongly potentiate insulin secretion [11,34,67,68], we analyzed the effects of FSK and PMA both separately and simultaneously on the insulin secretion by islets from LP and NP animals in the presence of 8.3 mM glucose (Fig. 1). Therefore, food restriction causes changes in the levels of insulin gene expression and content, leading to alterations in glucose-stimulated insulin secretion [69].

The results of the present study showed the presence of crosstalk between the pathways of the A and C kinases on insulin secretion in both experimental groups, but this effect was lower in the LP group. Our results showed clear crosstalk between these two kinases in NP islets, suggesting the existence of a secretory synergism between these kinases during the insulin secretory process.

Conversely, simultaneous stimulation of LP islets with FSK and PMA promoted no further increase in insulin secretion, which remained at the same level as with PMA treatment alone. This observation suggests that the synergism between the PKC and PKA pathways may be disrupted in LP islets.

Having identified the disruption of secretory synergism between the PKA and PKC pathways in the LP group, we next investigated the activities of both enzymes by determining the expression levels of phospho-CREB (Ser-133) (Fig. 2A and B) and phospho-(Ser) PKC substrate (Fig. 3A and B), targets of phosphorylation by PKA and PKC, respectively. Our results showed that FSK increased phospho-CREB

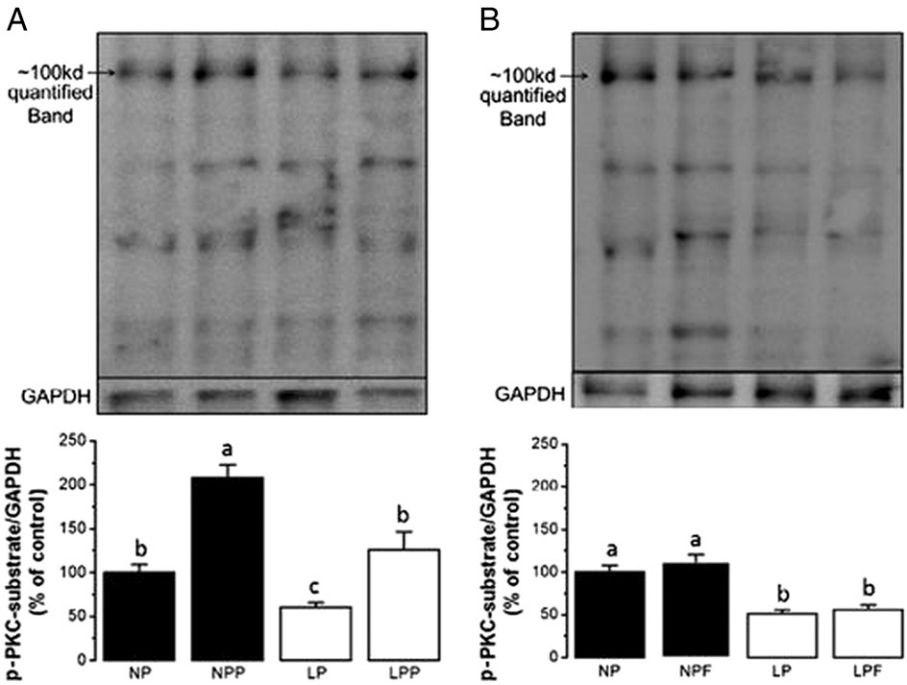

Fig. 3. A and B. Western blot analysis of phospho-(Ser) PKC substrate. Isolated islets from NP and LP rats were incubated in Krebs solution supplemented with 0.5% bovine albumin (m/v), FSK (10 μM) and PMA (200 nM) in the presence of glucose (8.3 mM) for 4 h. The columns are the means±S.E.M., n=5, from 2 independent experiments. Different letters indicate a significant difference (P<.05) according to 2-way ANOVA. NPF=NP+10 μM FSK; LPF=LP +10 μM FSK; NPP=NP+200 nM PMA; LPP=LP+200 nM PMA.

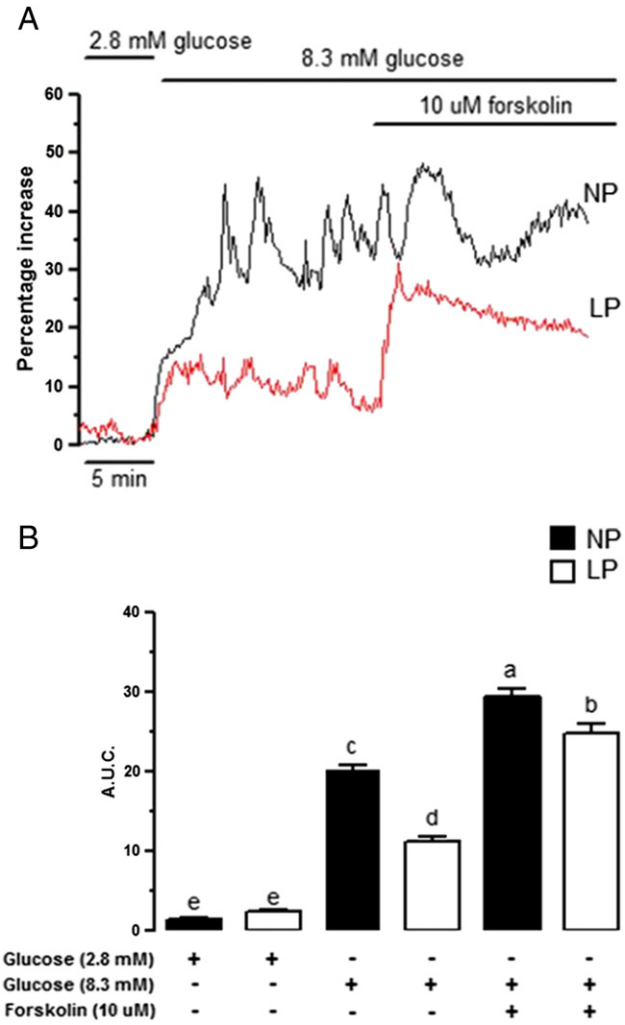

Fig. 4. Effects of glucose and FSK on Ca^{2+} oscillations. (A) Representative curves of the effects of 2.8 mM or 8.3 mM of glucose and FSK (10 μM) on transient rise in Ca^{2+} followed by oscillations in NP (black line) and LP (red line) in intact single islets. The basal level of each group was subtracted to compare the percentage increase in $[Ca^{2+}]_i$ oscillations. (B) Areas under the curve (AUC, $F_{340/380}$.min) showing the global Ca^{2+} entry in response to FSK. Values are the ratio of F340/F380 recorded for each group. Data are expressed as means±S.E.M.; n=5 independent experiments. Different letters indicate a significant difference (P<.05) according to ANOVA.

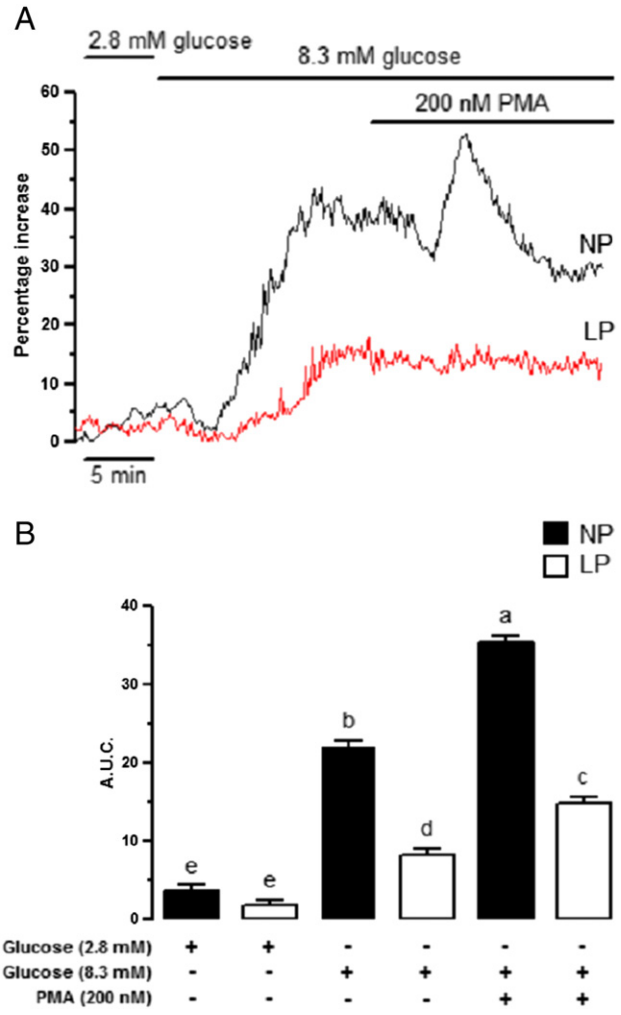

Fig. 5. Effects of glucose and PMA on Ca^{2+} oscillations. (A) Representative curves of the effects of 2.8 mM or 8.3 mM of glucose and PMA (200 nM) on transient rise in Ca^{2+} followed by oscillations in NP (black line) and LP (red line) in intact single islets. The basal level of each group was subtracted to compare the percentage increase in $[Ca^{2+}]_i$ oscillations. (B) Areas under the curve (AUC, $F_{340/380}$.min) showing the global Ca^{2+} entry in response to PMA. Values are the ratio of F340/F380 recorded for each group. Data are expressed as means±S.E.M.; n=5 independent experiments. Different letters indicate a significant difference (P<.05) according to ANOVA.

(Ser-133) expression in both groups, although to a lesser extent in the LP group (Fig. 2A), showing a reduction in the phosphorylation of CREB (Ser-133) in this group.

In addition, we analyzed the expression of phospho-CREB (Ser-133) in the presence of a PMA to verify whether the PKA pathway was activated by the PKC pathway and, if so, to verify the possibility of crosstalk between these two signaling pathways. The results of this study revealed an increase in the expression of phospho-CREB (Ser-133) in both groups when exposed to PMA, but this effect was smaller in the LP group (Fig. 2B). These results suggest that protein restriction disrupted the activity, and consequently the crosstalk, between PKC and PKA signaling in LP islets. These findings may be explained by the fact that PKC activation, independent of increases in intracellular Ca^{2+} levels, can phosphorylate several isoforms of AC, which would increase the cAMP levels as described in the literature [31]. This cAMP increase would both stimulate PKA activity and improve CREB phosphorylation, as was shown in our results.

By contrast, FSK did not alter the levels of phospho-(Ser) PKC substrate in either group (Fig. 3B). Thus, it is possible to deduce that PKC activation may regulate the activation of PKA substrates, whereas PKA activation does not seem to be directly involved in the activation of the PKC pathway or, therefore, in the insulin secretory crosstalk shown in the present study.

With respect to $[Ca^{2+}]_i$, our results (Figs. 4B and 5B) are consistent with the literature regarding the global Ca^{2+} entry reduction in rodents under protein restriction [9,41,42]. Surprisingly, the transient rise of Ca^{2+} in the presence of FSK in LP islets was higher than in the NP group (Fig. 4A). However, the LP islets were still unable to match the insulin secretion of the NP group (Fig. 1), possibly because the analysis of the AUC, which gives an estimation of the global Ca^{2+} entry, was lower in LP compared with NP islets (Fig. 4B).

In addition, cAMP can also modulate VDCCs in beta-cell membranes, thus leading to an increase in the level of cytosolic $[Ca^{2+}]_i$ [70–73]. Therefore, the reduction in global Ca^{2+} entry in LP islets may be explained by the reduction in cAMP observed in the present study.

The level of cytosolic $[Ca^{2+}]_i$ is directly proportional to the concentration of the Ca^{2+}-calmodulin complex (Ca^{2+}-Cal) [74]. This complex, in turn, activates Ca^{2+}-calmodulin kinases (CAMKs), leading

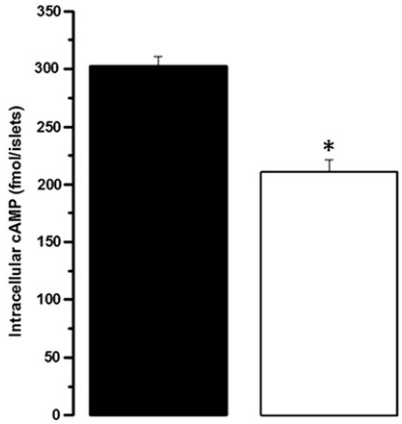

Fig. 6. cAMP levels. Quantification of cAMP levels in isolated islets of rats incubated in medium containing 8.3 mM of glucose and FSK (10 μM). The columns are the means± S.E.M.; n =10 independent experiments. *Different from NP islets (P<.05) using Student's t test for independent samples.

to the phosphorylation of CREB at the Ser-133 residue [75–77]. Experiments show that membrane depolarization and subsequent Ca^{2+} influx activates CREB by inducing CREB phosphorylation at Ser-133 [35]. In addition, CREB phosphorylation can be blocked by the chelation of Ca^{2+} in other tissues [78]. Furthermore, previous studies showed that glucose-induced Ca^{2+} influx activated the transcription factor CREB, which was required for glucose homeostasis and islet-cell survival, by regulating beta-cell gene expression, including expression of the insulin gene [79]. Thus, based on the literature and our results, we can infer that PKC activation increased cytosolic $[Ca^{2+}]_i$, which in turn increased the levels of the Ca^{2+}-Cal complex and led to activation of CAMKs, which in turn phosphorylated CREB at Ser-133 more efficiently in the NP group. These findings at least partially explain the crosstalk observed between the PKC and PKA signaling pathways and the reduced phosphorylation of CREB following PKC activation in the LP group.

An increase in cytosolic $[Ca^{2+}]_i$ is required to initiate insulin secretion, but the activation of PKA and PKC is required for a sustained secretory response [10]. Moreover, when PKC and PKA are activated simultaneously, the crucial ability of glucose to augment insulin secretion can be observed by the absence of an increase in $[Ca^{2+}]_i$ [45]. According to previous studies [10,45], we can infer that alterations in $[Ca^{2+}]_i$ may be a permissive but not a decisive factor in disrupting the crosstalk found in LP islets.

In summary, we conclude that protein restriction during early life causes several alterations in islets in rats fed a low-protein diet. The current study demonstrates, for the first time, that protein restriction causes a disruption in the crosstalk between the PKA and PKC signaling pathways and consequently in the secretory synergism. These changes can be primarily explained by the reduction in the activity of PKA and PKC and also by the reduction in the levels of cAMP, which leads to subsequent alterations in global Ca^{2+} entry.

Acknowledgments

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