FEBS Letters 445 (1999) 356–360 FEBS 21604

Redistribution of protein kinase C isoforms in rat pancreatic acini during lactation and weaning

E. Rodríguez-Martín[a], M.C. Boyano-Adánez[a], G. Bodega[b], M. Martín[b], C. Hernández[b], Y. Quin[c], M. Vadillo[d], E. Arilla-Ferreiro[a,*]

[a] *Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain*
[b] *Departamento de Biología Celular y Genética, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain*
[c] *UCB Pharma, Brussels, Belgium*
[d] *Centro Médico La Raza, México D.F., Mexico*

Received 13 November 1998; received in revised form 20 January 1999

Abstract Freshly enzymatically isolated pancreatic acini from lactating and weaning Wistar rats were used to investigate the role of protein kinase C (PKC) isoforms during these physiologically relevant pancreatic secretory and growth processes. The combination of immunoblot and immunohistochemical analysis shows that the PKC isoforms α, δ, and ε are present in pancreatic acini from control, lactating and weaning rats. A vesicular distribution of PKC-α, -δ, and -ε was detected by immunohistochemical analysis in the pancreatic acini from all the experimental groups. PKC-δ showed the strongest PKC immunoreactivity (PKC-IR). In this vesicular distribution, PKC-IR was located at the apical region of the acinar cells. No differences were observed between control, lactating and weaning rats. However, the immunoblot analysis of pancreatic PKC isoforms during lactation and weaning showed a significant translocation of PKC-δ from the cytosol to the membrane fraction when compared with control animals. Translocation of PKC isoforms (α, δ and ε) in response to 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) 1 μM (15 min, 37°C) was comparable in pancreatic acini from control, lactating and weaning rats. In the control group, a significant translocation of all the isoforms (α, δ and ε) from the cytosol to the membrane was observed. The PKC isoform most translocated by TPA was PKC-δ. In contrast, no statistically significant increase in PKC-δ translocation was detected in pancreatic acini isolated from lactating or weaning rats. These results suggest that the PKC isoforms are already translocated to the surface of the acinar cells from lactating or weaning rats. In addition, they suggest that isoform specific spatial PKC distribution and translocation occur in association with the growth response previously described in the rat exocrine pancreas during lactation and weaning.

© 1999 Federation of European Biochemical Societies.

Key words: Pancreatic acinus; Protein kinase C; Protein kinase C-δ; Immunohistochemistry; Lactation; Weaning

1. Introduction

During pregnancy and lactation, important changes occur in the pancreas. Hypertrophy of the pancreatic tissue is first seen during pregnancy and sustained during lactation, followed by hyperplasia late during the lactation period and the first 2 weeks after weaning [1]. In addition, pancreatic secretion is increased in lactating rats compared to control rats [2]. Recently, a considerable amount of evidence has become available supporting the role of protein kinase C (PKC) in the regulation of pancreatic acinar secretion [3,4] and proliferation [5]. However, it is not known whether the signalling cascades involving PKC are altered during lactation and weaning.

PKC is a family of phospholipid-dependent serine/threonine kinases which are involved in different cell signalling systems. To date, 12 different members have been identified and classified into three groups based on their structure and cofactor regulation: conventional PKCs (α, βI, βII and γ), novel PKCs (δ, ε, η, θ and μ) and atypical PKCs (ζ, λ and ι). All the isozymes require phosphatidylserine for their activation; however, only conventional PKCs are Ca^{2+}-dependent. Conventional and novel PKCs are activated by diacyglycerol (DAG) and phorbol esters [6,7]. The latter, potent tumor promoters, can substitute for DAG in activating PKC, suggesting that PKC plays some role in the process of neoplastic promotion of initiated tumor cells by phorbol esters. Activation of PKC has been proposed to be involved in a variety of cell responses, including cell proliferation, secretion, regulation of gene expression, membrane transport and smooth muscle contraction [8]. PKC activity has been detected in pancreatic acinar cells from several different species [4,9]. In the present study, we were interested in the hypertrophy and hyperplasia of pancreatic tissue that occurs during lactation and weaning as an example of the physiological regulation of pancreatic acinar growth. Since PKC is a key enzyme in secretory and proliferative processes, our aim was to test the hypothesis that pancreatic acinar proliferation and secretion that occurs during lactation and weaning could be attributed to similarly regulated events at the level of PKC. Since DAG has been implicated in pancreatic acinar secretory and proliferative processes [3,5], our attention was focused on the DAG-dependent isozymes such as the α, δ and ε PKC isoforms [3]. To investigate the functional differentiation of PKC isoforms during lactation and weaning, we investigated the distribution of PKC isoforms (α, δ and ε) in rat pancreatic acini during lactation and weaning by immunoblot and immunohistochemistry using isoform-specific antibodies.

2. Materials and methods

2.1. Materials

3,3′-Diaminobenzidine (DAB), PKC polyclonal antibodies (α, δ and ε) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) were purchased from Sigma Química (Madrid, Spain). Horseradish peroxidase (HRP)-conjugated affinity-purified secondary antibody was purchased from Chemicon International Inc. (Temecula, CA, USA). All other agents were of the highest purity commercially available.

*Corresponding author. Fax: (34) (1) 885 45 85.
E-mail: bqeaf@bioqui.alcala.es

0014-5793/99/$19.00 © 1999 Federation of European Biochemical Societies. All rights reserved.
PII: S0014-5793(99)00133-7

2.2. Experimental animals

A population of 16 newborn female Wistar rats were selected at birth and maintained until the age of 3 months. All animals were allowed access to a commercial diet and drinking water ad libitum, in a room maintained at a temperature of 20–22°C with a 12 h:12 h light/dark schedule. At the age of 3 months, 10 female rats were selected at random for impregnation, while the others were designated unmated controls. The presence of spermatozoa in the vaginal smear the following morning was regarded as evidence of impregnation. The day of parturition was considered day 0 of lactation. Lactation was permitted for 21 days following delivery. Eight animals (five nursing rats and three controls) were killed by decapitation after the third week of lactation and the rest the second week after weaning, since pancreatic tissue hyperplasia occurs late during lactation (third week after birth) and reaches a maximum at the end of the second week post lactation (fifth week after birth) [1].

2.3. Preparation of pancreatic acini

Wistar rats weighing 200–250 g and fed ad libitum were killed by decapitation. The pancreas was removed and trimmed free of fat, connective tissue and lymph nodes. Acini were prepared using minor modifications of the technique developed by Amsterdam et al. [10] and Estève et al. [11]. Krebs-Ringer medium contained 24.5 mM HEPES, 115 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 12 mM $MgSO_4$, 0.5 mM $CaCl_2$, 5 mM glucose, 2 mM glutamine, 0.2% (w/v) bovine serum albumin (BSA), 2% (v/v) essential amino acid solution, and 1% (v/v) non-essential amino acid solution. This medium was adjusted to pH 7.4 and gassed with O_2+CO_2. The pancreas was injected with 4.16 ml of oxygenated Krebs-Ringer medium containing 0.2 units of collagenase/ml. The distended pancreas was fragmented and incubated at 37°C at 60–70 oscillations/min in a shaking water bath for 10 min. After this incubation, the medium was discarded and replaced by 4.16 ml of fresh medium. The pancreas was regassed with O_2+CO_2 and the incubation repeated. The tissue was washed with Krebs-Ringer medium. Pancreatic acini were dissociated by passing the tissue through pipettes. The acinar preparation was purified by centrifugation for 1 min at $100\times g$.

2.4. Preparation of subcellular fractions

Acini were incubated with or without TPA (1 μM) for 15 min at 37°C. The TPA concentration used in our study (1 μM) agrees with the dose used by other authors [3,13]. Unstimulated conditions refer to acini incubated in the absence of TPA. All of the PKC isoforms studied showed detectable translocation with 10 nM TPA. Translocation was maximal at a TPA concentration of 1 μM and 15 min after addition of the phorbol ester (Fig. 2). The reaction was stopped by placing the acinar suspension on ice followed by centrifugation at $100\times g$ for 1 min. The pellet was resuspended in extraction buffer (20 mM Tris-HCl pH 7.5, 250 mM sucrose, 6 mM EDTA, 1 mM DTT, 1 mM PMSF, 20 μg/ml leupeptin, 25 μg/ml aprotinin), then frozen and thawed twice in liquid nitrogen and a 37°C water bath. Samples were disrupted by ultrasonication for 1 min. The suspension was centrifuged at $100\times g$ for 5 min to sediment nuclei and zymogen granules. After centrifugation at $100\,000\times g$ for 60 min, the resulting supernatant, referred to as the cytosolic fraction, was stored at −80°C until use. The pellet was resuspended in extraction buffer containing 0.5% (v/v) Triton X-100 and extracted by agitation for 30 min at 4°C. The suspension was then centrifuged at $100\,000\times g$ for 60 min, and the supernatant containing the membrane fraction was placed in aliquots and stored at −80°C. Aliquots of the two fractions were taken for protein determination by the method of Bradford using BSA as a standard [12].

2.5. Immunoblot analysis

Samples of cytosolic and membrane fractions were added to SDS sample loading buffer (130 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.001% bromophenol blue), vortexed and boiled for 5 min. The dose-response curves for protein amount were checked and 25, 35 and 40 μg of protein were chosen for PKC-α, -δ and -ε analysis, respectively, according to the linear range of their respective curves. Samples were electrophoresed in 12% SDS-polyacrylamide gels and then electroblotted to 0.45 μm nitrocellulose membranes in blotting buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 20% methanol) overnight. The membranes were subsequently stained with red Ponceau, washed with distilled water, cut into thin strips and then rinsed for 5 min with TBS (20 mM Tris-HCl, pH 7.6, 137 mM NaCl). The blots were incubated at room temperature for at least 1 h with TTBS (TBS pH 7.6, plus 0.1% (v/v) Tween-20) and 5% dry skimmed milk to block non-specific binding. The membranes were then incubated with rabbit anti-PKC-α, -δ and -ε antibodies (Ab-1) appropriately diluted in TBS (1:25 000 for anti-PKC-α and 1:10 000 for anti-PKC-δ and -ε) for 2 h at room temperature. After washing once for 5 min with TTBS plus 5% dry skimmed milk and twice for 5 min with TBS, the blots were further incubated for 1 h 30 min at room temperature with a goat anti-rabbit IgG (Ab-2) conjugated to peroxidase-anti-peroxidase, appropriately diluted in TBS. After washing as previously described, the immunoreactive bands were visualized by using DAB (0.12%) plus H_2O_2 (0.06%) dissolved in TBS. The specificity of each PKC antibody (anti-PKC-α, -δ or -ε) was previously tested. No anti-PKC immunoreactivity (IR) was observed when primary antibodies were removed from the assay (data not shown). The same test was carried out for HRP-conjugated affinity-purified secondary antibody and no PKC-IR was detected at that time. The density of the bands was measured using an image analyzing system (MIP, Microm, Spain) provided with a suitable computer program.

2.6. Immunocytochemistry

Fragments of pancreas were sectioned into 350 μm slices and then incubated with or without TPA (1 μM) for 15 min at 37°C. The reaction was stopped by placing them on ice and the fragments were fixed 15 min in 1.5% formaldehyde buffered to pH 7.5. After fixation, the fragments were dehydrated in ethanol, processed for paraffin embedding and then cut into 8 μm thick sections.

Slides from the different experimental groups were deparaffinized. The blockage to avoid non-specific binding was carried out by incubating the slides with normal goat serum (diluted 1:30 in TBS) for 30 min. Slides were then incubated with rabbit anti-PKC-α, -δ and -ε antibodies (Ab-1) appropriately diluted in TBS overnight at 4°C. After 5 min washes in TBS, the sections were incubated for 1 h 30 min at room temperature with a HRP-conjugated goat anti-rabbit secondary antibody appropriately diluted in TBS. Peroxidase activity was revealed with 0.12% DAB with 0.06% H_2O_2 for 15–20 min. Slices were washed with distilled water, dehydrated in ethanol and mounted on Depex. Some sections were incubated with pre-immune serum at 1:30 dilution as the primary antiserum, others were incubated only with DAB (neither primary nor secondary antibodies); these control sections showed no immunoreactive product (data not shown). The analysis of the immunostained sections was carried out by capturing the image with a microscope coupled to a video camera.

2.7. Data analysis

Statistical comparisons of the immunoblotting data were analyzed by ANOVA and the Newman-Keuls *t*-test. Means among groups were considered significantly different when the *P* value was less than 0.05.

3. Results

A vesicular distribution of PKC-IR was detected for the α, δ and ε PKC isoforms in pancreatic acini from control, lactating and weaning rats. In this vesicular distribution, PKC-IR was located in the apical pole of the acinar cells. No differences in its distribution were observed during lactation or weaning compared with their respective controls. PKC-δ showed the strongest immunoreactivity. Incubation of the pancreatic fragments with 1 μM TPA showed a diffuse cytoplasmic distribution of the immunostaining in control, lactating and weaning rats (Fig. 1).

Western blot analyses were performed to confirm the specificity of the antibodies and to examine the localization of PKC isoforms in subcellular fractions (data not shown). In each case, only one major band was detected that was not observed in the absence of the primary antibody. Similar experiments performed in the absence of the anti-rabbit secondary antibody showed no PKC-IR (data not shown).

Fig. 2 illustrates a representative immunoblot of PKC-α

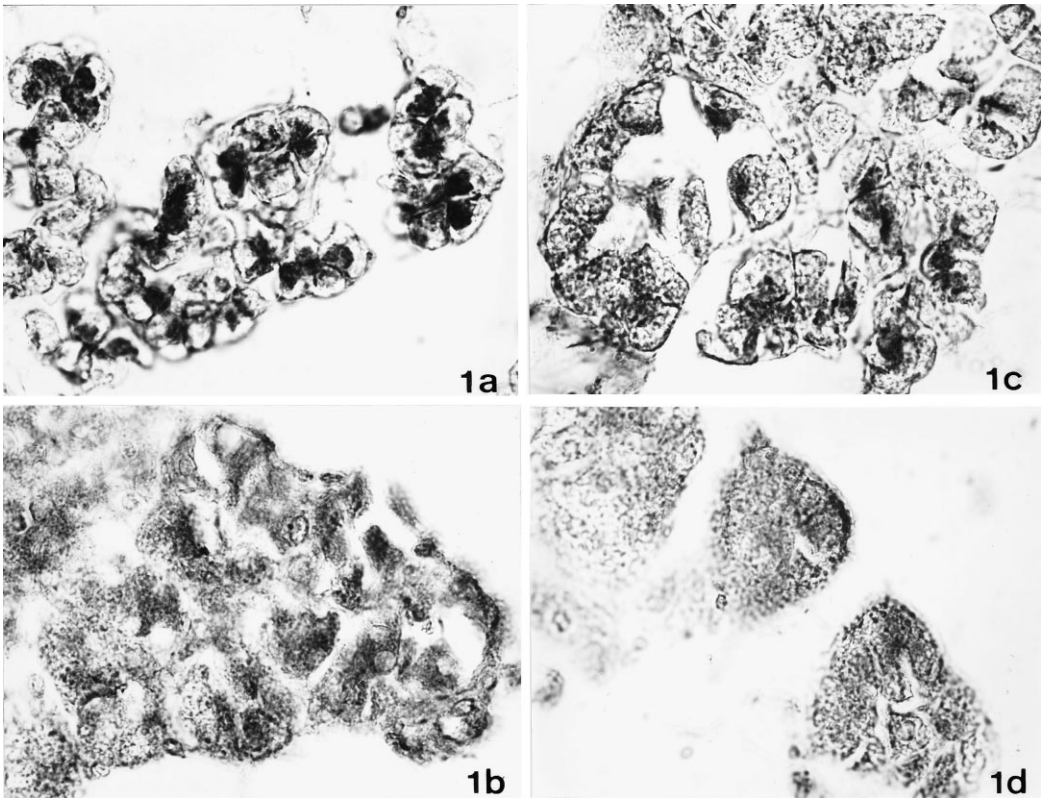

Fig. 1. Sections from rat pancreas immunostained with a rabbit polyclonal antibody against PKC-δ. Sections from control (a) and lactating (c) rat pancreas without TPA stimulation show vesicular staining for PKC-δ, predominantly localized to the apical pole of the acinar cells. Sections from control (b) and lactating (d) rat pancreas after TPA stimulation show a redistribution of PKC-immunoreactivity (PKC-IR) for PKC-δ (×404).

distribution in pancreatic acini. Similar results were observed for PKC-δ and -ε. In control acini, 30–35% of each isoform was associated with the membrane fraction.

Lactation and weaning led to PKC-δ translocation from the cytosol to the membrane. This translocation was higher at the late lactation period than at 2 weeks after weaning (Fig. 3). This effect was not observed, however, for PKC-α or PKC-ε.

To determine whether the change in PKC-δ distribution in pancreatic acini during lactation and weaning as compared with control animals could be due to an active translocation, the effect of TPA stimulation was also examined. Therefore, we studied the effect of TPA on the distribution of the PKC isoforms (α, δ and ε) in cytosol and membranes of pancreatic acini from control animals (Fig. 3).

The effect of TPA on the translocation of these PKC isoforms was dose-dependent. When pancreatic acini were incu-

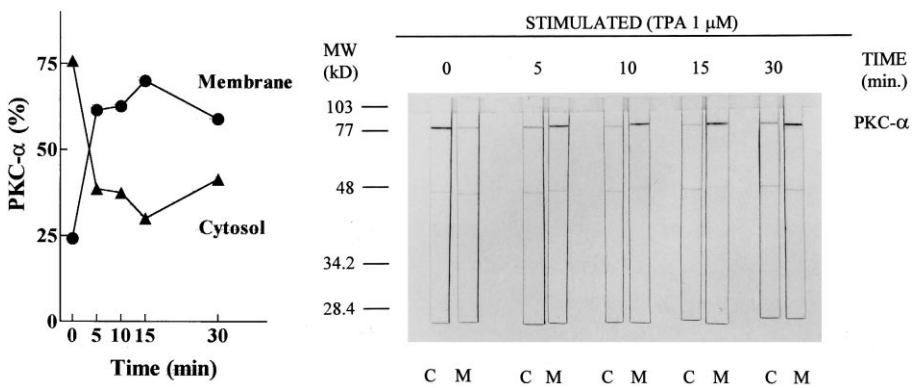

Fig. 2. Left panel: Effect of TPA on PKC-α distribution in cytosolic (C) and membrane (M) fractions from pancreatic acini. Acini were incubated with 1 μM TPA at 37°C for the indicated periods of time. PKC-α distribution was subsequently determined in membrane and cytosolic fractions by immunoblotting. The results are expressed as a percentage of the total amount of isoform present in both fractions. Results given are the means from three separate experiments. Right panel: Representative immunoblot analysis of PKC-α distribution in cytosol and membrane fractions of pancreatic acini incubated with TPA. Pancreatic acini were incubated with 1 μM TPA for 15 min at 37°C and the cytosolic and membrane fractions were subsequently analyzed for PKC-α immunoreactivity. The numbers on the left of the strips refer to the position of the molecular weight markers.

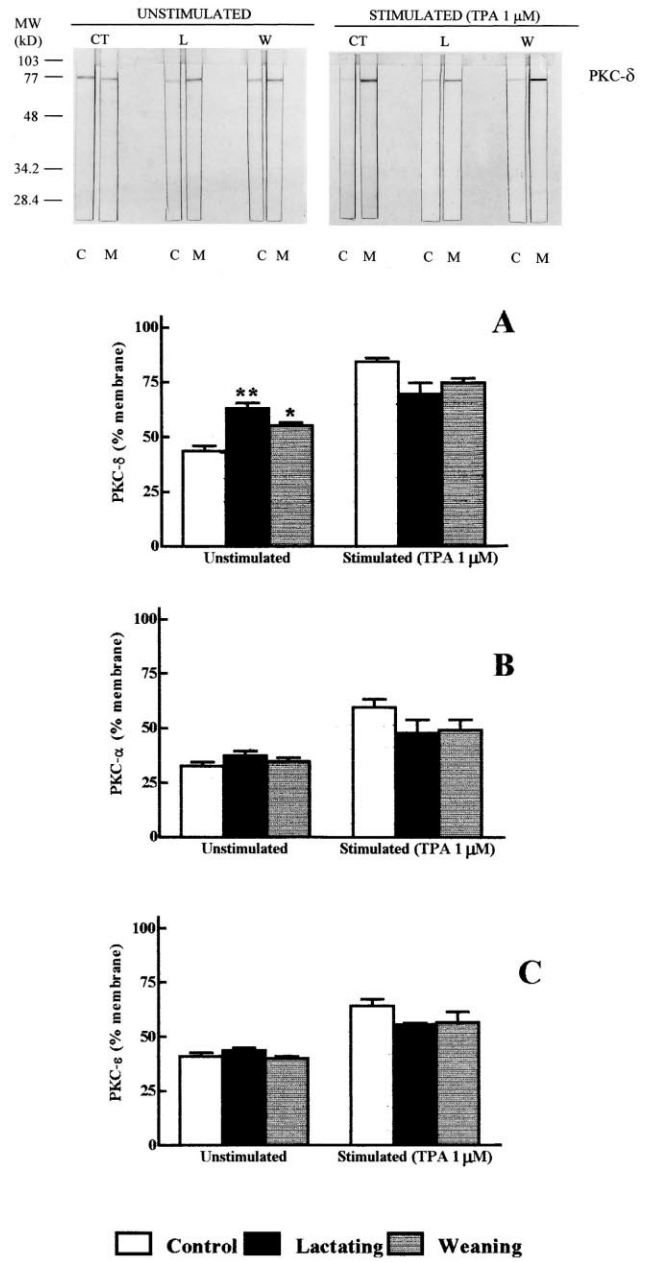

Fig. 3. Top panel: Representative immunoblot analysis of PKC-δ in pancreatic acini from control (CT), lactating (L) and weaning (W) rats. Acini were incubated for 15 min at 37°C with (stimulated) or without (unstimulated) 1 μM TPA and PKC-δ was then determined in cytosolic (C) and membrane (M) fractions by immunoblotting. Bottom panel: Effect of in vitro TPA on the distribution of PKC-δ, -α and -ε in membrane fractions of pancreatic acini isolated from control, lactating and weaning rats. Cytosol and membrane fractions were isolated and assayed for PKC immunoblot analysis as described in Section 2. Results given are the means from three separate experiments. Statistical comparison vs. unmated females: $*P < 0.05$, $**P < 0.01$.

bated with 10 nM TPA at 37°C, a detectable translocation occurred. Increasing TPA concentrations induced an increase in this translocation, reaching a maximum at the highest dose tested (1 μM) (data not shown). The TPA-induced PKC-α, PKC-δ and PKC-ε translocation was also time-dependent. Fig. 2 shows the immunoblot analysis for PKC-α. The TPA (1 μM) time course study revealed a time-dependent increase of PKC-α in the membrane fraction, with a maximum at 15 min after addition of TPA to the incubation medium. At this time period, a decrease in the cytosolic fraction was observed. Similar results were obtained for the PKC-δ and PKC-ε isoforms in control rat pancreatic acini (data not shown).

4. Discussion

This study demonstrates the presence of the PKC isoforms-α, -δ and -ε in pancreatic acini from control, lactating and weaning rats. The immunohistochemical results demonstrate a vesicular distribution of PKC-α, -δ and -ε in acinar cells. These three isoforms are present in the apical region of these cells. The detection of PKC-ε in the apical region of pancreatic acinar cells agrees with a previous study reported by others [13]. However, our study also detected the presence of PKC-α and -δ as opposed to the previously cited study. These differences may reflect the use of different antisera. The relationship of PKC with vesicles has previously been demonstrated [14,15] and it has been reported that PKC activity is essential for the formation of constitutive transport vesicles at the *trans*-Golgi network (TGN) [16,17]. In fact, PKC inhibitors prevent vesicle generation [18]; however, phorbol ester-induced PKC activation has been shown to induce vesicle accumulation [19] by inhibiting exocytosis [20]. This vesicle accumulation could be related to PKC-dependent annexin II phosphorylation [21]. Taking all this into account, it seems obvious that PKC plays an important role in the regulation of exocytosis, as has been previously suggested [22]. The present study shows a vesicular pattern for PKC-α, -δ and -ε. In addition, redistribution of PKC in the acinar cells is observed after TPA treatment. This redistribution may be due to an increase in vesicular synthesis and/or to changes in vesicular distribution. Because of the high amount of PKC associated with vesicles, the activation of cytosolic PKC may have only a marginal effect on this PKC-IR redistribution. In this sense, one may to hypothesize the existence of two different pools of PKC in pancreatic acinar cells, one constitutively bound to the membrane fraction (to the vesicles) and the other susceptible to translocation. In agreement with this idea, it was not possible to detect a 100% translocation because there is always PKC in the particulate (membrane) fraction. However, it must be noted that, for immunoblotting studies, zymogen granules (vesicles) were eliminated. In this case, redistribution could be due to vesicle accumulation and disorganization.

It is generally thought that the cellular distribution of PKC reflects the biological activity of the enzyme [23]. In the resting cells, PKC is usually localized in the cytosol as an inactive form. Upon stimulation of the cell, the enzyme is activated and generally translocated to a particulate fraction. In this study, immunocytochemical analysis showed that lactation and weaning leads to PKC-δ translocation from the cytosol to the membrane of pancreatic acini. This distribution of PKC-δ in the pancreatic acinar cell of lactating and weaning rats might be a unique sort of PKC translocation or may simply be due to the fact that the amount of PKC-δ expressed in these acinar cells is greater than that expressed in control cells. This apparent sustained translocation of PKC-δ might be due to endocrine changes that occur during lactation and weaning.

If endocrine factors are the cause of physiological changes

of the exocrine pancreas during lactation, several possible hormones could be responsible for the results obtained. Lactation is a condition associated with elevated prolactin levels [24]. An enhancement of cell proliferation by hyperprolactinemia has been described in the exocrine pancreas [25]. In addition, prolactin significantly increases total PKC activity [26]. Several lines of evidence suggest that cholecystokinin and gastrin are also trophic factors in the pancreas [27]. In addition, plasma levels of these two hormones are increased during lactation [28]. Both hormones also increase total PKC activity [29,30]. Therefore, these endocrine changes could explain, at least in part, the increase of PKC-δ translocation detected during lactation and weaning. Changes in PKC activity and subcellular distribution have also been described during lactation in rat mammary tissue [31–33].

The finding that PKC-δ was apparently translocated in a sustained chronic manner was rather surprising, given the fact that prolonged activation with phorbol esters generally leads to down-regulation and depletion of PKC from the cells [9,34]. However, Assert et al. [35] have recently shown an up-regulation of PKC-δ by phorbol esters in human T84 cells. Our present finding that TPA can induce translocation of PKC isoforms from the cytosol to the membrane fraction in pancreatic acini agrees with previous findings by others [3,36]. The fact that a statistically significant TPA-induced PKC-δ translocation was not detectable in pancreatic acini from lactating and weaning rats in contrast to control rats suggests that PKC-δ is already translocated to the membrane in pancreatic acini from lactating and weaning rats. In view of the antiproliferative effects of PKC-δ in several cell types [37–39], its sustained translocation may thus represent a protective mechanism against proliferative effects caused by cellular overactivation.

Acknowledgements: The authors thank Lilian Puebla from the Department of Biochemistry of Alcalá University for her linguistic assistance. This study was supported by a grant from the Dirección General de Investigación Científica y Técnica of Spain (PM95-0041).

References

[1] Jolicoeur, L., Asselin, J. and Morisset, J. (1980) Biomed. Res. 1, 482–488.
[2] Barrowman, J.A. and Mayston, P.D. (1973) J. Physiol. Lond. 229, 41p–42p.
[3] Pollo, D.A., Baldassare, J.L., Honda, T., Henderson, P.A., Talkad, V.D. and Gardner, J.D. (1994) Biochim. Biophys. Acta 1224, 127–138.
[4] Wooten, M.W. and Wrenn, R.W. (1984) FEBS Lett. 171, 183–186.
[5] Wooten, M.W. and Wrenn, R.W. (1985) Cancer Res. 45, 3912–3917.
[6] Ribera, J. and Beaven, M.A. (1997) in: Protein Kinase C (Parker, P.J. and Dekker, L.V., Eds.), pp. 133–166, R.G. Landes, Austin, TX.
[7] Casabona, G. (1997) Prog. Neuro-Psychopharmacol. Biol. Psychiatry 21, 407–425.
[8] Verme, T.B., Velarde, R.T., Cunningham, R.M. and Hootman, S.R. (1989) Am. J. Physiol. 257, G548–G553.
[9] Sung, C.K., Hootman, S.R., Stuenkel, E.L., Kuroiwa, C. and Williams, J.A. (1988) Am. J. Physiol. 254, G242–G248.
[10] Amsterdam, A., Salomon, T.E. and Jamieson, J.D. (1978) Methods Cell Biol. 20, 361–378.
[11] Estève, J., Vaysse, N., Susini, C., Fourmy, D., Pradayrol, L., Wunsch, E., Moroder, L. and Ribet, A. (1983) Am. J. Physiol. 245, G208–G216.
[12] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[13] Bastani, B., Yang, L., Baldassare, J.J., Pollo, D.A. and Gardner, J.D. (1995) Biochim. Biophys. Acta 1269, 307–315.
[14] Burgoyne, R.D. (1990) Annu. Rev. Physiol. 52, 647–659.
[15] Zhang, L., Dekorver, L. and Kolb, H. (1992) J. Neurocytol. 21, 833–847.
[16] Epand, R.M., Stafford, A.R. and Lester, D.S. (1992) Eur. J. Biochem. 208, 327–332.
[17] Westermann, P., Knoblich, M., Maier, O., Lindschau, C. and Haller, H. (1996) Biochem. J. 320, 651–658.
[18] Simon, J., Ivanov, I.E., Adesnik, M. and Sabatini, D.D. (1996) J. Cell. Biol. 135, 355–370.
[19] Goode, N.T., Hajibagheri, M.A.N. and Parker, P.J. (1995) J. Biol. Chem. 270, 2669–2673.
[20] Sapin, C., Baricault, L. and Trugnan, G. (1997) Exp. Cell Res. 231, 308–318.
[21] Johnstone, S.A., Hubaishy, I. and Waisman, D.M. (1992) J. Biol. Chem. 267, 25976–25981.
[22] Burgoyne, R.D. (1991) Biochim. Biophys. Acta 1071, 174–202.
[23] Baldassare, J.J., Henderson, P.A., Burns, D., Loomis, C. and Fisher, G.J. (1992) J. Biol. Chem. 267, 15585.
[24] Williamson, D.H. (1986) Reprod. Nutr. Dev. 26, 597–603.
[25] Matsuda, M., Mori, T., Park, M.K., Yanaihara, M. and Kawashima, S. (1994) Eur. J. Endocrinol. 130, 187–194.
[26] Banerjee, R. and Vanderhaar, B.K. (1992) Mol. Cell. Endocrinol. 90, 61–97.
[27] Seva, C., Scemama, J.L., Pradayrol, L., Sarfati, P.D. and Vaysse, N. (1994) Regul. Peptides 52, 31–38.
[28] Eriksson, M., Björkstrand, E., Smedh, U., Alster, P., Matthiesen, A.-S. and Uvnäs-Moberg, K. (1994) Acta Physiol. Scand. 151, 453–459.
[29] Linden, A., Eriksson, M., Hansen, S. and Uvnäs-Moberg, K. (1990) J. Endocrinol. 127, 257–263.
[30] Lichtenberger, L.M. and Trier, J.S. (1979) Am. J. Physiol. 237, E98–E105.
[31] Connor, K. and Clegg, R.A. (1993) Biochem. J. 291, 817–824.
[32] Caufield, J.J. and Bolander, F.F.J. (1986) J. Endocrinol. 109, 29–34.
[33] Foncea, R., Varela, S., Sapag-Hagar, M. and Lavandero, S. (1995) Res. Commun. Mol. Pathol. Pharmacol. 87, 253–268.
[34] Edervenn, A.G.H., Van Emst-De Vries, S.E., De Pont, J.J.H.H.M. and Willems, P.H.G.M. (1991) Eur. J. Biochem. 195, 679–683.
[35] Assert, R., Schatz, H. and Pfeiffer, A. (1996) FEBS Lett. 388, 195–199.
[36] Winand, J., Poloczek, P., Delporte, C., Moroder, L., Svoboda, M. and Christophe, J. (1991) Biochim. Biophys. Acta 1080, 181–190.
[37] Watanabe, T., Ono, Y., Taniyama, Y., Hazama, K., Igarashi, K., Ogita, K., Kikkawa, U. and Nishizuka, Y. (1992) Proc. Natl. Acad. Sci. USA 89, 10159–10163.
[38] Mischak, H., Goodnight, J.A., Kolch, W., Martiny-Baron, G., Schächtele, C., Kazanietz, M.G., Blumberg, P.M., Pierce, J.H. and Mushinski, J.F. (1993) J. Biol. Chem. 268, 6090–6096.
[39] Hirai, S., Izumi, Y., Higa, K., Kaibuchi, K., Mizuno, K., Osada, S., Suzuki, K. and Ohno, S. (1994) EMBO J. 13, 2331–2340.