Volume 295, number 1,2,3, 110–112 FEBS 10523 December 1991

Gene expression of type I phospholipase A_2 in pancreatic beta cells

Regulation of mRNA levels by starvation or glucose excess

S. Metz[1], D. Holmes[2], R.P. Robertson[3], W. Leitner[2] and B. Draznin[2]

[1]*Divisions of Endocrinology and Departments of Medicine of the University of Wisconsin and Middleton Memorial Veterans Hospital, Madison, WI, USA,* [2]*Denver VA Medical Center, Denver, CO, USA* and [3]*University of Minnesota, Minneapolis, MN, USA*

Received 10 October 1991

Messenger RNA from intact rat pancreatic islets, or from transformed hamster beta (HIT) cells, hybridized with the cDNA probe for type I (but not type II) phospholipase A_2. The levels of phospholipase A_2 mRNA increased in islets from fasted rats; they decreased in islets cultured in a high glucose concentration (control values at 5.5 mM glucose = 150±6% of those at 22 mM) which impaired subsequent insulin secretion (reduction in second-phase release = 70±11%). These studies uniquely demonstrate that type I phospholipase A_2 is expressed specifically in beta cells and that nutrient availability modulates transcript levels, an effect which could contribute to the detrimental influence of prolonged hyperglycemia on islet function.

Insulin; Pancreatic islet; Phospholipase; Glucose; Beta cell; Fasting

1. INTRODUCTION

Rat pancreatic islets contain one (or more) phospholipases of the A_2 type (PLA_2); furthermore, considerable data implicate PLA_2 in the regulation of insulin secretion through the mediation of the phospholipid hydrolysis products generated (i.e. arachidonic acid and lysophospholipids) (summarized in [1]). However, little is known about the type of PLA_2 present in endocrine cells. Phospholipases A_2 can be sudivided into two general groups [2] based on structural determinants. Type I PLA_2 is found in mammals, in the exocrine pancreas, lung, gastric mucosa, and spleen. The regulation of type I PLA_2 gene expression is unexplored. Type II PLA_2, in contrast, is present in platelets, inflammatory exudates, vascular smooth muscle, chondrocytes, synovial cells, spleen, platelets, mesangial cells and placenta. Using cDNA probes directed against type I or type II PLA_2, we examined pancreatic islets, transformed pancreatic beta cells, and other endocrine glands, to assess the regulation of PLA_2 mRNA levels in two states in which altered nutrient availability is known to perturb insulin release – fasting, and exposure to high glucose concentrations. The former inhibits PLA_2 activity [3] and impairs physiologic insulin release [4]. 'Glucotoxic' islets also have impaired insulin secretion [5]. These studies comprise the first data addressing the presence of the gene for phospholipase A_2, and the regulation of its expression, in a peptide-secreting endocrine gland.

Abbreviations: PLA_2, phospholipase A_2; cDNA, complementary DNA.

Correspondence address: S. Metz, H4/554 Clinical Science Center, Section of Endocrinology, 600 Highland Avenue, Madison, WI 53792, USA. Fax: (1) (608) 262 6743

2. MATERIALS AND METHODS

2.1. *Materials*

The cDNA probes for PLA_2 types I and II (cloned from cDNA libraries isolated from rat pancreas and spleen, respectively) were generously provided by Shionogi Research Laboratories (Osaka, Japan) and are described in detail in their publications [6–8]. The probe for mouse β-actin was provided by Dr. Bruce Spiegelman (Boston, MA). Deoxycytidine-5'-triphosphate (dCTP), tetratriethylammonium salt, [α-^{32}P]-, having a specific activity of 3000 Ci/mmol, was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA).

2.2. *Islet isolation and processing*

Islets, isolated from male Sprague–Dawley rats, as described [9], were sequentially handpicked on two separate occasions under stereomicroscopic control in order to rigidly exclude contamination by exocrine pancreatic cells [2]. For studies of the exposure to different glucose concentrations or to various test compounds, islets were cultured for 18–20 h in CMRL 1066 medium containing 5% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and the indicated test agents, and were suspended in 1 ml of 4 M guanidinium thiocyanate and kept at −70°C until further processing. RNA was obtained by centrifugation through cesium chloride [10]. For studies of whole pancreas, RNA was extracted and resuspended in the presence of 0.2% diethylpyrocarbonate to inhibit RNases active in that tissue [11]. A similar protocol was used to study dynamic insulin secretion from these islets except that 10% fetal calf serum was present overnight; 100 islets were placed into each chamber and perifused, using a previously described procedure [12], in Krebs-Ringer bicarbonate buffer, for 5 min at 4.4 mM glucose, followed by 76 min at 27.5 mM glucose. Effluent medium was collected every 1 min for 15 min and then every 2 min. Transformed Syrian hamster beta cells were maintained in culture in RPMI 1640 medium as previously described in detail [13].

 Published by Elsevier Science Publishers B.V.

2.3. *Northern blot analysis*

Approximately equal amounts of denatured total cellular RNA (5–15 μg per lane) was electrophoresed on 0.8% agarose and 2.7% formaldehyde gels, blotted onto nitrocellulose membranes, and cross-linked by baking at 80°C for 2 h in a vacuum oven. DNA probes for PLA_2 type I and II, and for β-actin, were labeled with [^{32}P]dCTP by the random-primer method or nick translation using Boehringer-Mannheim labeling kits. Prehybridization (and hybridization) was carried out in 0.007 M Tris-HCl, pH 8.0, containing 10% dextran sulfate, 40% deionized formamide, 4 × SSC, 0.8 × Denhardt's solution, and 20 μg/ml denatured salmon-sperm DNA, for 16 h at 42°C; ^{32}P-labeled cDNA was added during hybridization. The hybridized blots were washed 4 times in 2 × SSC and 0.1% SDS for 5 min at room temperature and subsequently twice in 0.5 × SSC and 0.1% SDS for 30 min at 55°C. Autoradiographic exposures of the blots to Kodak X-Omat AR film were carried out at −70°C using two intensifying screens. The levels of RNA transcripts were measured by vertically scanning autoradiograms with a video densitometer (Bio-Rad, model 620). Gene transcript concentrations are expressed as the densitometric ratio for each band of PLA_2 gene transcript compared to the value for the corresponding β-actin peak. HIT cell RNA was analyzed on 1.5% agarose-formaldehyde gels and transferred to a nylon hybridization membranes (Micron Separations, Westford, MA) by electroblotting.

3. RESULTS

A strong signal for type I PLA_2 (transcript size: 0.95 kb) was consistently found using freshly isolated or overnight-cultured rat islets (Fig. 1); no signal for type II PLA_2 was detected. A similar signal was detected in studies using HIT cells (Fig. 1). No signal for either type of PLA_2 was detected in adrenal glands, testes, or unfractionated pituitary glands (40 μg of RNA from 1–3 pituitaries was applied to each lane). In rats fasted for 48 h, islet levels of PLA_2 (type I) mRNA increased from control values of 0.59±0.16 to 1.49±0.32 (n=5 experiments, mean ± SE; P<0.05). Refeeding was associated with values which had returned towards control (0.96±0.19; n=3). As expected [2], unfractionated pancreas (which is overwhelmingly comprised of exocrine tissue) also expressed type I PLA_2. However, in sharp contrast to the findings with islets, fasting actually *reduced* PLA_2 signal to 63±5% of levels in pancreases from fed rats (control = 3.49±1.02; fasted = 2.43±0.82; n=7 experiments; P=0.01).

Compared to islets cultured for 18–20 h at 5.5 mM glucose, islets cultured at 22 mM glucose had reduced PLA_2 mRNA levels in each of 6 separate paired experiments (high glucose culture: 2.00±0.88; normal glucose culture: 3.04±0.65; P<0.05). Such treatment did not alter first phase (0–7 min) insulin release; however, in each of 5 paired studies, second phase secretion (8–60 min), expressed as the incremental areas above basal levels, was reduced by 70±11% (Fig. 2) from 15 772±4411 to 4535±1535 μU (P<0.05).

In certain non-endocrine cells which synthesize a secretory, type II PLA_2, gene expression is augmented by cyclic AMP or interleukins [14,15]. However, culture of islets with 200 μM dibutyryl cyclic AMP (control

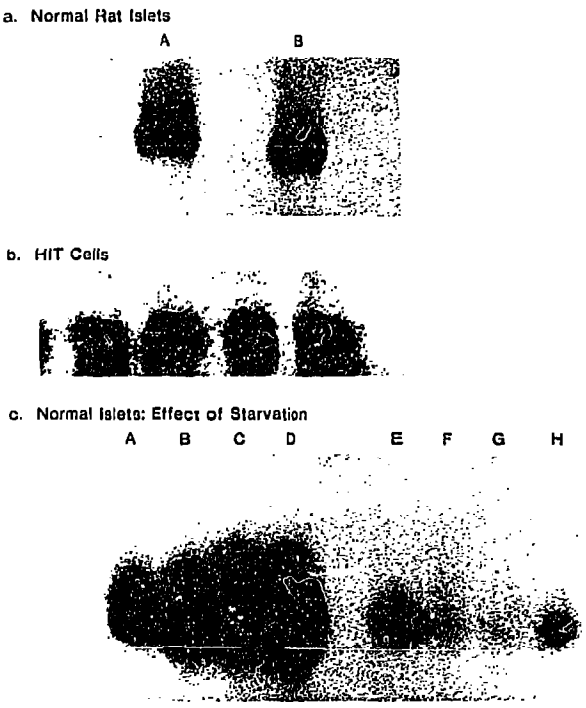

Fig. 1. Detection of type I phospholipase A_2 mRNA in insulin-secreting tissues. (a) Normal rat islets (lane A contains RNA from 1800 islets, lane B from 2800 islets). (b) HIT cells from passages 71, 80, 87 and 98. (c) Normal rat islets after 48 h fasting (lanes A–D) vs. islets from fed rats (lanes E–H).

1.38±0.13; cAMP: 1.60±0.22; n=4 pairs; P=NS) or 5 μM forskolin (control: 1.64±0.03; forskolin: 1.34±0.07; n=3) did not significantly alter PLA_2 mRNA.

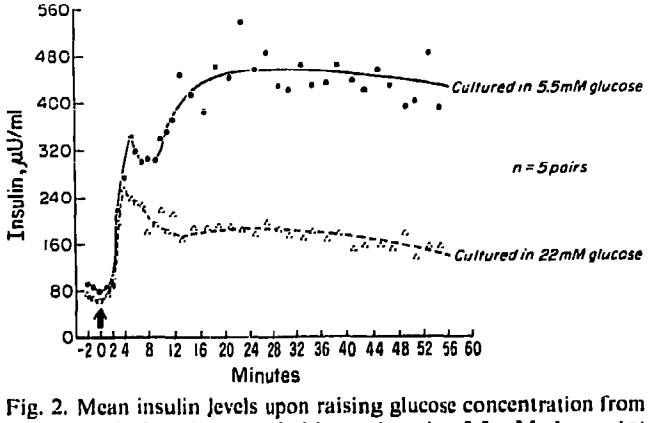

Fig. 2. Mean insulin levels upon raising glucose concentration from 4.4 mM to 27.5 mM (arrow) in islets cultured at 5.5 mM glucose (●) or at 22 mM (△). Basal insulin levels averaged 86±15 and 52±6 μU/ml, respectively, and were not significantly different between the two conditions.

4. DISCUSSION

These data establish, for the first time, type I PLA_2 gene expression in an endocrine gland (islets of Langerhans). Although type I PLA_2 is expressed in pancreatic exocrine tissue ([2] and current studies), contamination with such cells does not explain the findings with islets since the latter were twice hand-picked to exclude all acinar tissue. Furthermore, the PLA_2 mRNA levels were regulated in a diametrically-opposed fashion in the two tissues, with fasting increasing them in the endocrine pancreas and decreasing them in the exocrine pancreas. Additionally, the presence of the message in HIT cells demonstrates that beta cells in particular express PLA_2; indeed, PLA_2 activity is demonstrable not only in intact adult rat islets [16] but in cultured monolayers of neonatal rat islet cells [17,18] which are comprised (90–98%) of beta cells. No signal for type II PLA_2 was seen: this indicates the specificity of our findings, since types I and II PLA_2 share a degree of sequence homology [19].

Forty-eight hours of fasting markedly increased PLA_2 message. Since PLA_2 activity is reportedly decreased by starvation [3], we speculate that the former observation reflects a compensatory effect on transcription (or mRNA stability) in response to an impairment of PLA_2 activity by fasting. For example, fasting reduces Ca^{2+} influx into islets [20]; cytosolic Ca^{2+} levels are a major determinant of PLA_2 activity [16].

An area of much current interest is the ability of subacute or chronic elevations in ambient glucose levels to impair signal transduction in the beta cell, i.e. 'glucose toxicity' [5,21]. This lesion is probably not simply explained by a defect in glucose transport or metabolism [22]. The reduction of PLA_2 mRNA levels suggests that an impairment of PLA_2 activity might play a role in 'glucose toxicity'; desensitization to glucose is associated with an inhibition of the activity of another phospholipase (phospholipase C) as well [23]. In addition to direct effects of glucose, elevated ambient insulin levels, accumulating in response to high glucose levels, could be involved mechanistically, since high insulin levels have been suggested to impede phospholipase activity [24] and any subsequent insulin release [25]. Insulin has also been reported to reduce type II PLA_2 expression in non-endocrine cells [26]. Future studies correlating PLA_2 protein levels, PLA_2 activity and insulin release will be required to address this novel hypothesis. Likewise, elucidation of the site of nutrient action (e.g. on transcription or on the stability of mRNA) and of the molecular mechanisms involved will require additional investigations.

Acknowledgements: These studies were supported by grants from the National Institutes of Health (DK37312, DK38325) and by the Department of Veterans Affairs. The technical assistance of Mary Rabaglia is gratefully acknowledged. The authors are grateful to Drs. Sakata, Okamoto and Arita for supplying the cDNA probes for PLA_2.

REFERENCES

[1] Metz, S.A. (1988) Am. J. Med. 85 (Suppl. 5a) 9–21.
[2] Heinrikson, R.L., Krueger, E.T. and Keim, P.S. (1977) J. Biol. Chem. 252, 4913–4921.
[3] Tadayyon, M., Bonney, R.C. and Green, I.C. (1990) J. Endocrinol. 124, 455–461.
[4] Buchanan, K.D., Vance, J.E. and Williams, R.H. (1969) Metabolism 18, 155–162.
[5] Davalli, A.M., Ricordi, C., Socci, C., Brahgi, S., Bertuzzi, F., Fattor, B., DiCarlo, V., Pontiroli, A.E. and Pozza, G. (1991) J. Clin. Endocrinol. Metab. 72, 202–208.
[6] Sakata, T., Nakamura, E., Tsuruta, Y., Tamaki, M., Teraoka, H., Tojo, H., Ono, T. and Okamoto, M. (1989) Biochim. Biophys. Acta 1007, 124–126.
[7] Ishizaki, J., Ohara, O., Nakamura, E., Tamaki, M., Ono, T., Kanda, A., Yoshida, N., Teraoka, H., Tojo, H. and Okamoto, M. (1989) Biochem. Biophys. Res. Commun. 162, 1030–1036.
[8] Ohara, O., Tamaki, M., Nakamura, E., Tsuruta, Y., Fujii, Y., Shin, M., Teraoka, H. and Okamoto, M. (1986) J. Biochem. 99, 733–739.
[9] Metz, S.A. (1986) Diabetes 39, 808–817.
[10] MacDonald, R.J., Swift, G.H., Przybyla, A. and Chirgwin, J.M. (1987) in: Guide to Molecular Cloning Techniques (Berger, S.L. and Kimmel, A.R. eds.) Methods Enzymol. vol. 152, pp. 219–227. Academic Press, San Diego.
[11] Han, J.H., Statowa, C. and Rutter, W.J. (1987) Biochemistry 26, 1617–1625.
[12] Mehler, P.S., Sussman, A.L., Maman, A., Leitner, J.W. and Sussman, K.E. (1980) J. Clin. Invest. 66, 1334–1338.
[13] Zhang, H.-J., Walseth, T.F. and Robertson, R.P. (1989) Diabetes 38, 44–48.
[14] Nakano, T., Ohara, O., Teraoka, H. and Arita, H. (1990) FEBS Lett. 261, 171–174.
[15] Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F. and Vosbeck, K. (1991) Biochem. J. 273, 199–204.
[16] Laychock, S.G. (1982) Cell Calcium 3, 43–54.
[17] Dunlop, M.E. and Larkins, R.G. (1984) Biochem. Biophys. Res. Commun. 120, 820–827.
[18] Metz, S.A. and Dunlop, M. (1990) Biochem. Biophys. Res. Commun. 167, 61–66.
[19] Kanda, A., Ono, T., Yoshida, N., Tojo, H. and Okamoto, M. (1989) Biochem. Biophys. Res. Commun. 163, 42–48.
[20] Vara, E., Fernández-Martin, O., Garcia, C. and Tamarit-Rodriques, J. (1988) Diabetologia 31, 687–693.
[21] Dunbar, J.D., Houser, F. and Levy, J. (1989) Diabetes Res. Clin. Pract. 7, 187–196.
[22] Colella, R.M., May, J.M., Bonner-Weir, S., Leahy, J.L. and Weir, G.C. (1987) Metabolism 36, 335–337.
[23] Zawalich, W.S., Zawalich, K.C., Shulman, G.I. and Rossetti, L. (1990) Endocrinology 126, 253–260.
[24] Verspohl, E.J., Ammon, H.P.T. and Klosa, M. (1990) Biochem. J. 267, 339–342.
[25] Draznin, B., Goodman, M., Leitner, J.W. and Sussman, K.E. (1986) Endocrinology 118, 1054–1058.
[26] Craven, P.A., Patterson, M.C. and DeRubertis, F.R. (1988) Diabetes 37, 429–435.