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FEBS Letters 343 (1994) 137–140

FEBS 13933

FEBS LETTERS

Prolactin treatment increases GLUT2 but not the G protein subunit content in cell membranes from cultured neonatal rat islets

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Received 22 February 1994

Abstract

Neonatal rat islets exhibit a reduced secretory response to glucose, compared to adult rat islets. The maturation of the secretory response is stimulated by prolactin (PRL). We show here by immunoblot analysis that PRL increases the β-cell/liver glucose transporter GLUT2 in membrane fractions from cultured neonatal rat islets. This increase (+86%) may explain, at least in part, the development of a mature glucose response. G proteins modulate insulin secretion from pancreatic β-cells. We show here by immunoblot analysis that, in contrast to the effect on GLUT2, PRL treatment does not modify the G protein subunits αi2, αi3, αo, αs, αq and β35 and β36, in cultured neonatal islets.

Key words: Islet; Neonatal rat; GLUT2; G protein; Prolactin

1. Introduction

Fetal and neonatal rat islets exhibit a reduced secretory response to glucose, compared to adult rat islets [1–4]. The maturation of the secretory response is influenced by many factors, including fuel metabolites, neurotransmitters and hormones. Among these hormones, prolactin (PRL) plays an important role in the maturation of the glucose sensing mechanisms. In fact, PRL treatment affects several physiological parameters of pancreatic β-cell, such as the glucose-induced reduction of K^+ permeability [4], the glucose stimulation threshold [5], cell-to-cell coupling [5], [^{3}H]thymidine incorporation [6] and the proliferation of β-cells in vitro [7].

GLUT2, the liver/βcell glucose transporter, plays a key role in the glucose sensing apparatus [8]. One of the two goals of this study was to check if PRL treatment modifies glucose sensing in neonatal islets through an effect on GLUT2.

G proteins are important modulators of pancreatic islet function. Pertussis toxin ADP-ribosylates G protein α subunits in pancreatic islet membranes [9,10]. Adrenalin, somastotatin, galanin and prostaglandin inhibit insulin secretion through G protein modulated pathways [9]. Somatostatin is used to regulate β-cell function in human neonates with persistent hyperinsulinemic hypoglycemia [11]. cAMP augments insulin secretion [12] and is modulated by the cholera toxin sensitive G protein, Gs. Glucose induces an increase in phosphatidylinositol breakdown [12], as do the phospholipase C (PLC) isoenzymes. Some PLC isoenzymes are under the control of G protein αq subunit, some under the control of G protein βγ subunits and some under the control of Pertussis Toxin sensitive G proteins [13–15]. Any modification in these G protein modulated cascades could theoretically contribute to alter glucose sensing. The second goal of this study was to check if PRL treatment could modify the G protein-modulated cascades in neonatal islets. For that purpose we quantified some of the known G protein subunits by immunoblot analysis.

We show here that PRL treatment of cultured neonatal islets for 7–9 days increases the GLUT2 content in membranes of islets, but that the content of G protein subunits (αi2, αi3, αo, αs, αq and β35 and β36) are unaltered by PRL treatment.

2. Materials and methods

2.1. Islets isolation and culture

Islet from neonatal rats (2- to 36-h-old) were obtained as described [4] and maintained in culture at 37°C under 5% CO_2. The culture medium consisted of Eagle's Minimum Essential Medium (Biofluids, Rockville, MD) supplemented with 2 mM glutamine, 10% fetal calf serum, 10 mM glucose, 100 IU/ml penicillin and 100 μg/ml streptomycin. Half of the plates also contained 2 μg/ml ovine PRL (highly purified, isolated by Dr. AF Parlow, Harbor-University of California-Los

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Abbreviations: DTT, dithiotreitol; EGTA, (ethylene glycol-bis[β-aminoethyl ether]-*N,N,N'N'*-tetraa cetic acid; NEM, *N*-ethylmaleimide; PMSF, phenylmethylsulfonil fluoride; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; TTBS, tris-bufferd saline.

SSDI 0014-5793(94)00318-P

Angeles Medical Center and, kindly provided by the National Hormone and Pituitary Program of the NIDDK). Culture medium was changed every second day. After 7–9 days in culture, the medium was discarded and the islets were used for membrane preparation.

2.2. Membrane preparation

The islets from 4 different preparations were pooled and were suspended (10% v/v) in Tris-HCl 10 mM pH 7.9 containing DTT 1 mM, NaCl 10 mM, glycerol 20%, EGTA 1 mM, PMSF 10 μM, leupeptin 0.1 mg/ml, aprotinin 10 μg/ml, *p*-aminobenzamidine 1 mM, and soy bean trypsin inhibitor 1 mg/ml; sonicated at 4°C (two pulses of 15 s) and centrifuged at 1000 × *g* for 10 min; and the supernatant was centrifuged 25 min at 16,000 × *g*. The pellet was resuspended in the same buffer as above and alkylated with NEM as follows.

2.3. NEM treatment

Membranes were incubated for 10 min at 85°C in the presence of SDS 0.75% and DTT 10 mM. NEM was then added to get the following final concentrations: NEM 40 mM, SDS 0.6%, DTT 8 mM and membrane protein slightly less than 1 mg/ml. After 2 h at 4°C, 1 vol of 2 × Laemmli buffer [16] containing 10% β-mercaptoethanol was added. Protein concentration was checked with the Amido-black method [17] prior to loading onto the gel.

2.4. Immunobloting

NEM-treated membrane proteins were resolved on a SDS-polyacrylamide gel (0.1% SDS, 11% acrylamide, 0.08% bisacrylamide, 75 μg protein/lane). Proteins were then transferred to PVDF membranes (Millipore, 150 mA 20 h) in Tris 25 mM glycine 192 mM buffer containing 20% methanol. PVDF membranes were blocked for 2 h at room temperature in TTBS containing 2.5% gelatin and immunoblotted (20 h at room temperature) with the antisera (GLUT2 antiserum 1/1000, SW antiserum 1/200, other antisera were affinity purified and used at 2–5 μg/ml in TTBS-gelatin 2.5%). The PVDF membranes were washed with TTBS and the antibody–antigen complex was detected by ^{125}I-labeled protein A (Amersham, 0.2 μCi/ml in TTBS-gelatin 2.5% for 1–2 h). Antibodies specific for G protein carboxy-terminal domains (see Table 1) were either purchased from NEM-Dupont or kindly donated by Dr P.K. Goldsmith and A.M. Spiegel, NIH, Bethesda, MD.

3. Results

We performed an immunoblot analysis of neonatal islets with an antibody specific for the liver/β-cell glucose transporter GLUT2. As can be seen from Fig. 1, this antibody labelled a 53 kDa band corresponding to GLUT2 in both control and PRL treated islets. PRL treatment induced a marked increase in GLUT2 content of neonatal islets (+86%), as appreciated by quantification with a phosphorimager, Molecular Dynamics).

We also performed an immunoblot analysis of neonatal islets membranes using antibodies specific for various G proteins subunits. As can be seen from Fig. 2, AS, specific for the G protein carboxy-terminal domain common to αi1 and αi2 detected one single band of 40 kDA in both control and PRL-treated islets. This band corresponds to αi2, since it co-migrated with the lower band of brain cholate extract (data not shown), which has been identified as αi2 [18,19]. Moreover, LD, an antibody specific for an internal decapeptide of αi1, failed to detect any α subunit in neonatal islets (data not shown). PRL treatment left αi2 content unchanged (Fig. 2). We also studied other G proteins subunits, namely αi3, αo, αs, αq and β35 and β36 with EC, GC2, RM, QL and SW antibodies. The G protein subunit contents detected by these antibodies were also unchanged by PRL treatment (Fig. 2).

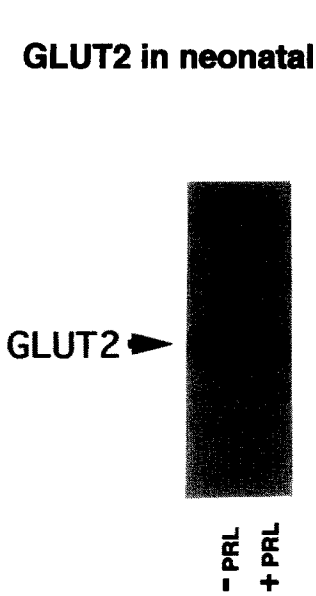

Fig. 1. Effect of PRL on GLUT2 protein measured by immunoblot. Neonatal islets were cultured for 7–9 days in Eagle's Minimal Essential Medium in the absence or presence of 2 μg/ml of PRL. Neonatal islets protein (75 μg/lane) were analyzed by immunoblotting with antiserum to GLUT2. This is one representative blot out of two.

Discussion

The first step in glucose-induced insulin secretion is the entry of the sugar into the β-cells. This is mediated by the glucose transporter GLUT2, located at the β-cell plasma membrane. Alterations in the GLUT2 expression in β-cells have been implicated in the altered secretory response to glucose in rat NIDDM and IDDM models [20,21]. Furthermore, different tumoral cell lines, such β-TC, HIT and RIN cells with an abnormal response to glucose show reduced amounts of GLUT2, compared to normal β-cells [22]. According to these authors, the elevated level of GLUT1, with a lower K_m for hexose transport than GLUT2, in HIT cells could account for the abnormal secretory response to lower concentrations of glucose.

Table 1
G protein subunit antibodies

Antibody	Domain	Sequence*	G protein subunit
AS	C-terminal	KENLKDCGLF	αi1, αi2
EC	C-terminal	KNNLKECGLY	αi3
RM	C-terminal	RMHLRQYELL	αs
QL	C-terminal	QLNLKEYNLV	αq, α1 1
GC2	N-terminal	GCTLSAEERAALERSK	αo
SW	C-terminal	SWDSFLKIWN	β35, β36

* Single-letter amino acid code.

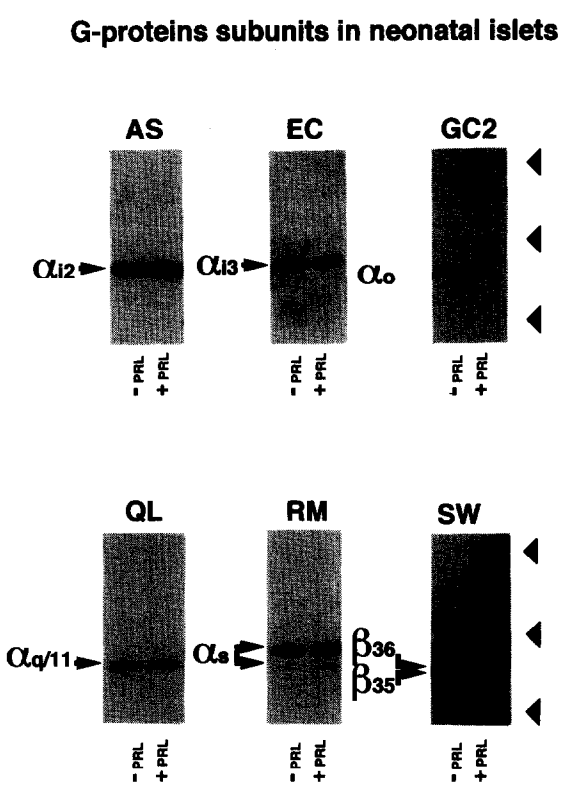

Fig. 2. Effect of PRL on αi2, αi3, αo, αq/11, αs and β35 and β36 G proteins subunits measured by immunoblot. Neonatal islets were cultured as indicated in the legend of Fig. 1. Islets proteins (75 μg/lane) were analyzed by immunobloting with antiserum AS, EC, GC2, QL, RM, SW to the above G proteins subunits, respectively. This is one representative blot of 2 or 3.

In neonatal islets the low secretory response to glucose has been ascribed to a diminished ATP production in response to a rise in glucose or leucine concentration compared to adult islets [23]. The recent findings that reduction in hexokinase IV activity may lead to some types of NIDDM [24] and that overexpression of yeast hexokinase in mouse β-cells decreases diabetes [25] support the idea that a reduction in the hexokinase activity plays a role in certain types of diabetes. However, alterations in glucokinase activity were not observed in neonatal islets [23]. On the contrary, at low glucose concentrations, the glycolysis rate is higher in neonatal than adult islets. However, the glycolysis rate increases to a lesser extent in neonatal than adult islets in response to a rise in glucose concentration [23]. These observations together with the fact that insulin secretion, in response to intermediate glucose concentrations (5.6–11.1 mM), was even higher in neonatal than in adult islets [2] rather indicate that the poor secretory response to high glucose concentrations could be linked to alterations in the content of glucose transporters of the GLUT2 type in islet cells.

Our data show that PRL treatment increases the GLUT2 content in the membranes from neonatal islets supporting the view that the maturation of the glucose sensing mechanism, induced by this hormone is owing to, at least in part, an effect on GLUT2 expression.

Recent data from this laboratory indicated that PRL potentiates the TPA/PKC-induced insulin secretion in the presence of glucose [4]. We investigated the possibility that PRL also affects this pathway upstream i.e. at G protein αq and βγ subunits levels: αq is an activator of the PLCβ1, and βγ are activators of the PLCβ2 [13–15]. Gαq is considered to be ubiquitous. We find that Gαq is also present in neonatal islets but unaffected by PRL treatment. Thus, the PRL effect on the PKC response is probably limited to a distal effect already described [4].

In conclusion these data clearly indicate that PRL treatment increases GLUT2 content in neonatal rat islets but does not alter the content of many G protein subunits.

GLUT2 deficiency has been implicated in the low response to glucose from tumoral β-cells, in diabetes and in fasting. This is the first report implicating that GLUT2 could play a critical role in the normal maturation response during the neonatal period of development.

Acknowledgements: This work was supported in part by Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPQ), and Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP). A.C.B. is a professor on leave from the Departamento de Fisiologia e Biofisica, UNICAMP, Campinas SP, Brazil. E.M.C. is a teacher assistant on leave from the Departamento de Educacao Fisica, UNESP 13500 Rio Claro, SP, Brazil.

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