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Effect of Hormones on Expression of Prolactin Receptor Messenger Ribonucleic Acids in Pancreatic Islets of Adult Female Mice *In Vitro*

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ABSTRACT—We studied the effects of hormones on expression of prolactin receptor (PRL-R) mRNA in pancreatic islets of adult female mice *in vitro*. We quantified mRNA expression in small amounts of the islet tissue by competitive PCR and one-sided competitive PCR. Fifty pancreatic islets from adult female mice were cultured in a well for 4 days with or without ovine prolactin (PRL), bovine growth hormone or estradiol-17β. PRL (1 μg/ml) significantly increased the insulin secretion and the amount of PRL-R mRNA relative to that of β-actin mRNA. Growth hormone (1 μg/ml) also increased the relative amount of PRL-R mRNA, although it did not significantly increase the insulin secretion. Neither insulin secretion nor the relative amount of PRL-R mRNA was affected by estradiol (100 ng/ml). The ratio of the short form to the long form of PRL-R mRNA was not altered by these hormones. The present observation that PRL increased PRL-R mRNA expression in pancreatic islets thus suggests the possibility that PRL up-regulates the tissue sensitivity to PRL itself during lactation.

INTRODUCTION

Prolactin (PRL) acts on isolated pancreatic islets, and stimulates the proliferation and insulin secretion of the islet B-cells in rodents and humans (Nielsen, 1982; Billestrup and Nielsen, 1991; Brelje and Sorenson, 1991; Brelje *et al.*, 1993). These effects of PRL may contribute to the marked hyperplasia of pancreatic islets during pregnancy and lactation (Nielsen, 1982; Green and Taylor, 1972) and furthermore, to a "homeorhetic" control of nutrition during lactation (Bauman and Currie, 1980; Matsuda *et al.*, 1996). Namely, it regulates the partitioning of nutrients to maintain lactation, partly through modification of the pancreatic endocrine function. Certain types of prolactin receptor (PRL-R) mRNAs, which have a common sequence encoding the extracellular domain, have been identified in several organs of mice (Davis and Linzer, 1989). Pancreatic islets also have more than two types of PRL-R mRNAs of which the expression is modified by PRL itself in the neonatal rat (Møldrup *et al.*, 1993). However, the small size of pancreatic islets and low number of their PRL receptors made it difficult to study fine changes in PRL-R expression by conventional analytical methods, except in rat insulinoma cells (Møldrup *et al.*, 1990; Asfari *et al.*, 1995). In this context, the use of the polymerase chain reaction (PCR) is promising since it allows one to detect and quantify low levels of mRNAs such as those of PRL-R (Nagano and Kelly, 1994).

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In this study, we quantified the complementary DNA (cDNA) encoding the extracellular domain of PRL-R and the ratio of the short form to the long form of the receptor (S/L ratio) by competitive PCR (cPCR) and one-sided cPCR (Matsuda and Mori, 1996), respectively. We then studied the effects of PRL, growth hormone (GH) or estrogen on expression of PRL-R mRNAs in primary cultures of pancreatic islets from adult female mice to determine whether or not these hormones modify the sensitivity of the islets to PRL during pregnancy and lactation.

MATERIALS AND METHODS

Islet isolation and culture

Pancreatic islets were isolated from 2-month-old female mice of the BALB/c strain (CLEA Japan, Inc., Tokyo) using collagenase (Moskalewski, 1965) with a slight modification. Mice were starved overnight, then killed by decapitation. The pancreas was excised immediately, transferred to ice-cold Hanks' medium and cut into small pieces with scissors. The pieces were placed in 1.5 ml of Hanks' medium containing 350 U collagenase (CLS IV; Worthington Biochemical Co., Freehold, NJ, USA) and vigorously shaken (200 strokes/min) at 37°C for 20 min. The digest was diluted with 10 ml of ice-cold Hanks' medium. After centrifugation at 400 × g for 1 min, the supernatant was discarded, and 250 μg DNase (DN-25; Sigma Chemical Co., St. Louis, MO, USA) was added. The digest was then transferred to a glass dish with ice-cold Hanks' medium, and the pancreatic islets were collected with a glass pipette under the binocular microscope and pooled in another dish filled with RPMI 1640 medium containing 10 mM glucose (GIBCO BRL, Gaithersburg, MA, USA) supplemented with 25 mM HEPES (pH 7.4). Groups of 50 islets were transferred to 24-well plates and cultured free-floating in 2 ml of RPMI

1640 medium containing 10 mM glucose, 15% fetal bovine serum (GIBCO), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C. After pre-incubation without hormones for 24 hr, the islets were cultured in media with or without hormones for 4 days. Under these culture conditions fibroblastic overgrowth was not seen even after 4 days of culture. Ovine PRL (oPRL), bovine GH (bGH) and estradiol-17β (E_2) were obtained from Sigma.

The culture medium was replaced with fresh medium every 24 hr, and stored at -70°C, until determination of insulin concentration by an enzyme immunoassay (Glazyme insulin EIA kit; Wako, Tokyo) as described previously (Matsuda *et al.*, 1994).

Preparation of cDNA samples

Total RNA was extracted from cultured pancreatic islets by the acid guanidinium-phenol-chloroform method (Chomczynski and Sacchi, 1987). Immediately after removing the culture medium, 0.5 ml of denaturing solution (4 M guanidinium thiocyanate, 0.5% sodium sarcosyl, 100 mM 2-mercaptoethanol, 25 mM sodium citrate, pH 7.0) containing 2 μg yeast tRNA as the carrier RNA was added to each well, then the islets were homogenized with Microson™ Ultrasonic Cell Disruptor MS-50 (Heat Systems Ultrasonics, Inc., Farmingdale, NY, USA) with 3/32" microprobe at output settings 12 for 3 × 5 s. The homogenate was mixed with 50 μl of sodium acetate (2 M, pH 4), and proteins and genomic DNA were removed after phenol-chloroform treatment. Total RNA was then precipitated with isopropanol.

After denaturation at 90°C for 5 min, the RNA sample was ice-chilled. Template cDNAs for PCR were generated from half of the sample by reverse transcription (RT) as described previously (Matsuda and Mori, 1996). A 20-μl reaction mixture containing 200 U Superscript™ reverse transcriptase (GIBCO BRL) and 100 nM oligo $d(T)_{12-18}$ (Pharmacia) as a primer was incubated successively at 23°C for 10 min, 42°C for 1 hr, 95°C for 10 min, then ice-chilled and divided into 4-μl aliquots.

Primers, competitors and standard DNAs for cPCR

Primers, competitors and standards for cPCR of mPRL-R and mouse β-actin (mBA) cDNA are shown in Fig. 1. Those for mPRL-R were prepared as described before (Matsuda and Mori, 1996).

Competitor and standard for cPCR of mBA cDNA were prepared as follows. The RT-PCR product generated from mouse liver RNA using mBAs-1 and mBAa-1 as the PCR primers was TA-subcloned into pT7Blue(R) (Novagen, Madison, WI, USA). A mutant mBA cDNA was generated by mutagenesis with PCR using mBAs-1 and mBAa-2B, and mBAs-2B and mBAa-1 as primers and subcloned into pT7Blue(R). These plasmids were digested with *Eco*R I and *Pst* I, and the inserts were purified by using glass beads. Their concentrations were estimated by absorbance at 260 nm (1 OD unit was estimated to be 50 μg/ml). The DNA which contains the sequence for the 3' part of mBA mRNA was used as the standard, and the DNA which was the same as the standard except for a *Bam*H I site instead of a *Kpn* I site was used as the competitor. Competitive PCR of mBA cDNA was performed using mBAs-1 and mBAa-1 as primers. The sequences of primers were as follows: mBAs-1; 5'-TCTAGACTTCGA-GCAGGAGATGGCC-3', mBAa-1; 5'-CTAGAAGCACTTGCGGTGCA-CGATG-3', mBAs-2B; 5'-CTGGTGGATCCACCATGTAC-3', mBAa-2B; 5'-GTGGATCCACCAGACAACAC-3' (*Bam*H I sites are underlined).

All restriction enzymes were products of Takara Shuzo Co., Ltd., Tokyo.

Competitive PCR and determination of the amount of target cDNAs

The cDNA standard was competitively amplified in a 40-μl reaction mixture containing PCR buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-Cl, pH 8.0), 200 mM each of dNTPs, and 500 nM each of primers, mPRLR-4 and mLTHR-1, or mBAs-1 and mBAa-1. Deoxycytidine 5'-[α-^{32}P]triphosphate ([α-^{32}P]dCTP; 148 kBq/tube) (Amersham, UK) was also added to the mixture to radio-label the products. The reaction mixture was overlaid with mineral oil (Sigma), and hot start PCR amplification proceeded in a thermal cycler (PTC-100; MJ Research, Inc., USA). After denaturation at 90°C for 5 min, 1 U of TaKaRa *Taq* DNA polymerase (Takara) was added to the reaction mixture, then 40 cycles of PCR were performed. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min. After the last extension, the reaction mixture was heated at 98°C for 10 min to denature the PCR product and inactivate DNA polymerase, and left at room temperature for the PCR product to randomly anneal while cooling.

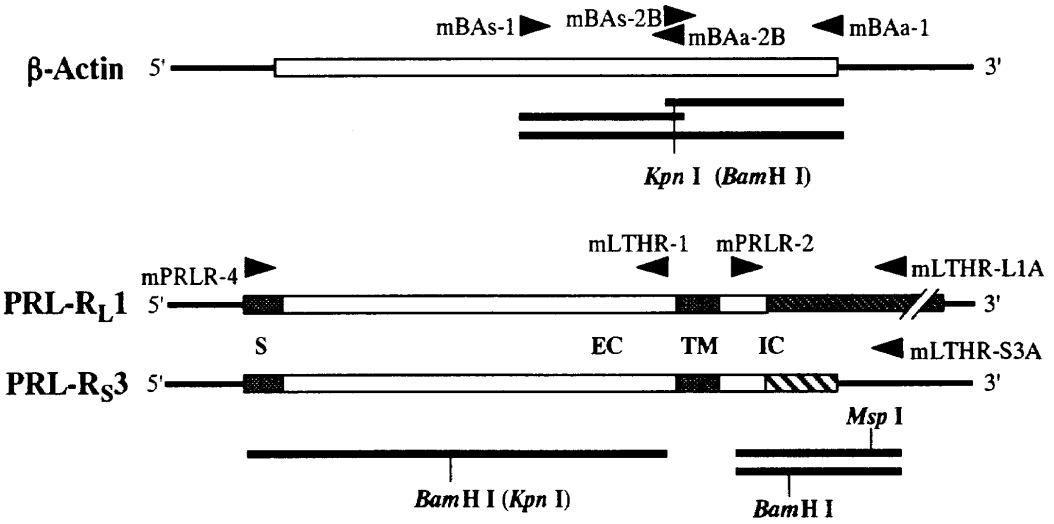

Fig. 1. Schematic representation of portions of mBA mRNA and two forms of mPRL-R mRNAs (PRL-R_L1 and PRL-R_S3) with PCR primers and the PCR products. Coding regions of the mRNAs are shown with boxes, PCR primers with arrowheads, and PCR products with bold lines. Each PCR product has an identical restriction site for *Bam*H I, *Kpn* I or *Msp* I as indicated. The two forms of mPRL-R mRNAs have a common sequence corresponding to the 5' noncoding region, signal peptide (S), extracellular domain (EC), transmembrane domain (TM) and a part of the intracellular domain (IC). They differ in the rest of the 3' coding and noncoding regions.

Two step cPCR amplifications were performed on cDNA samples to determine the amounts of both mPRL-R and mBA cDNAs in one tube at a time. The first amplification was performed in a 20-μl reaction mixture containing 5 nM each of the two pairs of primers by 40 cycles of PCR. An additional amplification by 20 cycles of PCR was carried out in a 40-μl reaction mixture containing 500 nM each of the two pairs of primers and [α-^{32}P]dCTP with 20 PCR cycles. The reaction mixture was heated at 98°C for 10 min and left at room temperature.

The PCR product was digested with *Bam*H I and/or *Kpn* I, resolved by 4% polyacrylamide gel electrophoresis in TBE buffer and stained with ethidium bromide. Gel strips containing each pair of PCR products cleaved by the restriction enzyme(s) was excised under UV light and transferred into a glass vial to measure Cerenkov radiation using a scintillation counter (LS6000IC; Beckman Instruments, Inc., Fullerton, CA, USA). PCR product without restriction enzyme digestion was also resolved by electrophoresis, and a gel strip equivalent to the cleaved fragments at the same position served as the background. The radiation intensity of the gel corresponding to the fragments cleaved by *Bam*H I or *Kpn* I was proportional to the amount of homodimers of the PCR product bearing the restriction site. Sometimes the gels were exposed to Kodak X-OMAT film after electrophoresis.

One-sided cPCR to determine the S/L ratio

The ratio of the short form of mPRL-R cDNA (PRL-RS3) to the long form (PRL-RL1), was determined by one-sided cPCR as described (Matsuda and Mori, 1996). Briefly, the cDNA was amplified by means of PCR using 100 μM of the common primer, mPRLR-2, and 500 μM each of identical primers, mLTHR-L1A and mLTHR-S3A (Fig. 1). The ratio of the two cDNAs was estimated from that of two PCR products which were distinguished from each other by the presence of unique restriction sites.

Statistical analysis

All results are expressed as the mean ± S.E. Statistical significance of difference between means was assessed with Student's *t*-test. The number of samples is expressed as 'n' in parenthesis.

RESULTS

Competitive PCR of the standard with the competitor

Mouse PRL-R standard DNA and the competitor were combined in various proportions so that the sum of both would be 10^5 molecules in each PCR-reaction tube. These samples were then amplified by cPCR. PCR amplification of mPRL-R generated a 653-bp product, a portion of which was cleaved by the appropriate restriction enzymes into fragments of 329 bp and 324 bp. Figure 2A shows the relationship between the initial ratio of the standard to the competitor and the ratio of the PCR product cleaved by *Bam*H I to that cleaved by *Kpn* I. The square root of the ratio of the cleaved PCR products corresponded to the initial ratio of the standard to the competitor. These findings demonstrated that both the standard and the competitor were amplified with equal efficiency, the PCR products annealed randomly and the restriction enzyme completely digested the homodimer PCR product bearing the restriction site. Thus, these observations enabled us to estimate the amount of mPRL-R cDNA by cPCR using a competitor bearing a single mutation.

We also established a method to estimate the amount of mBA cDNA by cPCR using a competitor bearing two point mutations (Fig. 2B). In this case, PCR amplification of mBA generated a 471-bp product, of which a portion was cleaved by the appropriate restriction enzymes into fragments of 248 bp and 223 bp.

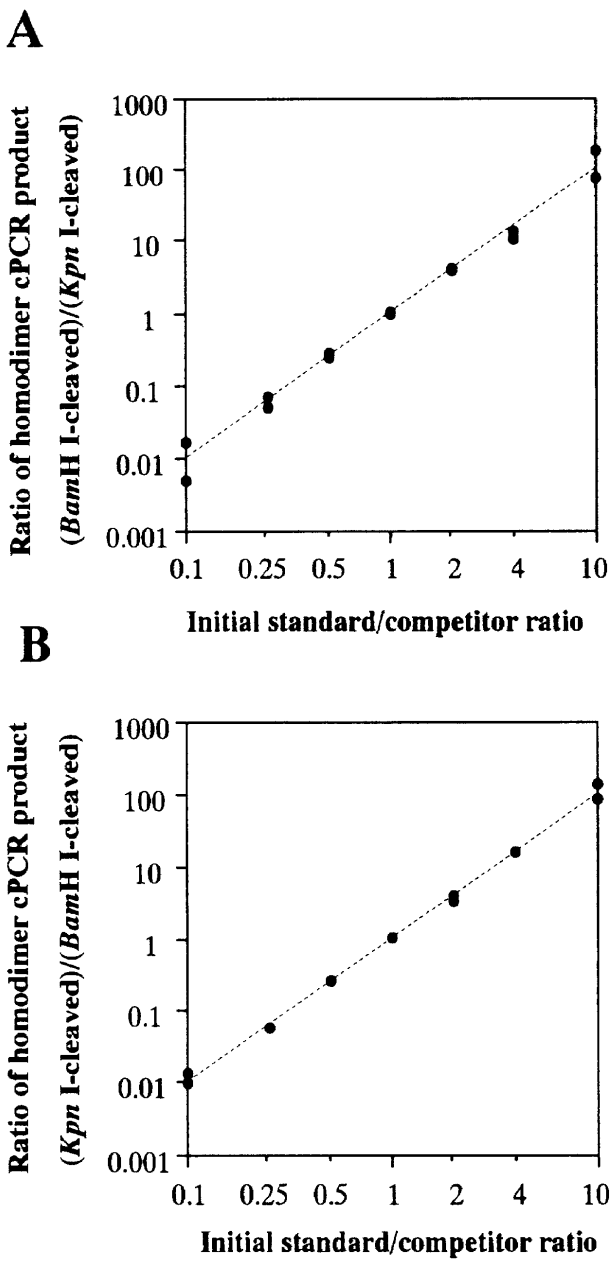

Fig. 2. Competitive PCR of the standard cDNAs with competitors. (**A**) A mixture of mPRL-R standard DNA and its competitor (the sum was about 10^5 molecules per tube) combined in various proportions was amplified by cPCR. Note that the ratio of PCR product cleaved by *Bam*H I to that cleaved by *Kpn* I nearly equals the square of the initial ratio of the standard to the competitor. Two sets of the experiment were performed. (**B**) A mixture of mBA standard DNA and its competitor (the sum was about 10^7 molecules per tube) combined in various proportions was amplified by cPCR. The ratio of PCR product cleaved by *Kpn* I to that cleaved by *Bam*H I nearly equals the square of the initial ratio of the standard to the competitor. Two sets of the experiment were performed.

Competitive PCR of a cDNA sample with the competitor

We performed cPCR amplification of both mPRL-R and mBA of a pancreatic islet cDNA sample to ensure that the

method could allow us to estimate the amount of mPRL-R and mBA cDNAs. After cDNA was made from about 200 islets and was divided into 20 aliquots, serially diluted competitors were added to each aliquot. Competitive PCR amplification was performed, then the amounts of mPRL-R and mBA cDNAs were estimated. Figure 3A shows an example of cPCR product after cleavage with restriction enzymes and gel electrophoresis. Using the two step amplification, nearly the same amount of the two PCR products corresponding to mPRL-R and mBA were obtained with low background by cPCR (data not shown). The square root of the ratio of the PCR products cleaved by each restriction enzyme was proportional to the amount of the competitors added to the reaction tube (Fig. 3B, C). These results demonstrated that the amounts of both mPRL-R and mBA cDNA in pancreatic islet cDNA could be estimated from the ratio of the cPCR products cleaved by the restriction enzymes at least when the ratio of the target cDNA to the competitor was between 0.25 and 4.

Effect of hormones on the amount of PRL-R mRNA in cultured islets

We examined the effects of oPRL, bGH or estrogen on the ratio of mPRL-R to mBA in cultured islets by cPCR. Complementary DNA from 50 islets was divided into 5 aliquots, mixed with both 10^5 or 10^6 molecules of the competitor for mPRL-R and 10^7 or 10^8 molecules of the competitor for mBA, then the amount of those cDNA was determined. The results indicated that mPRL-R and mBA cDNAs ranging from 4×10^4 to 9×10^5 and from 7×10^6 to 5×10^7 molecules, respectively, were generated from about 10 islets. The ratio of mPRL-R to mBA was increased significantly by adding oPRL (1 μg/ml) or bGH (1 μg/ml) to the culture medium, but the ratio was not affected by E_2 (100 ng/ml) (Fig. 4).

S/L ratio of PRL-R mRNA in cultured islets

The S/L ratio was examined by one-sided cPCR, in pancreatic islets cultured with or without hormones. Figure 5A shows a one-sided cPCR product digested by *Bam*H I which cleaves the short form and/or *Msp* I which cleaves the long form derivative. Long form mPRL-R mRNA was dominant in the pancreatic islets. Adding oPRL, bGH or E_2 to the culture medium did not affect the S/L ratio (Fig. 5B).

Effect of hormones on insulin secretion in cultured islets

The concentration of insulin was measured in islet culture media to which hormones were added (Fig. 6). Four days of culture with oPRL significantly increased the insulin secretion. Bovine GH tended to stimulate insulin secretion, but the difference from the control was not statistically significant. Insulin secretion was not affected by E_2.

DISCUSSION

This paper describes the stimulatory effects of PRL and GH on expression of PRL-R mRNA in pancreatic islets from adult female mice. Pregnancy and lactation are accompanied by an increase in circulating levels of GH, PRL or placental lactogen (PL), marked hyperplasia of the pancreatic islet cells (Green and Taylor, 1972; Parsons *et al.*, 1992), as well as an

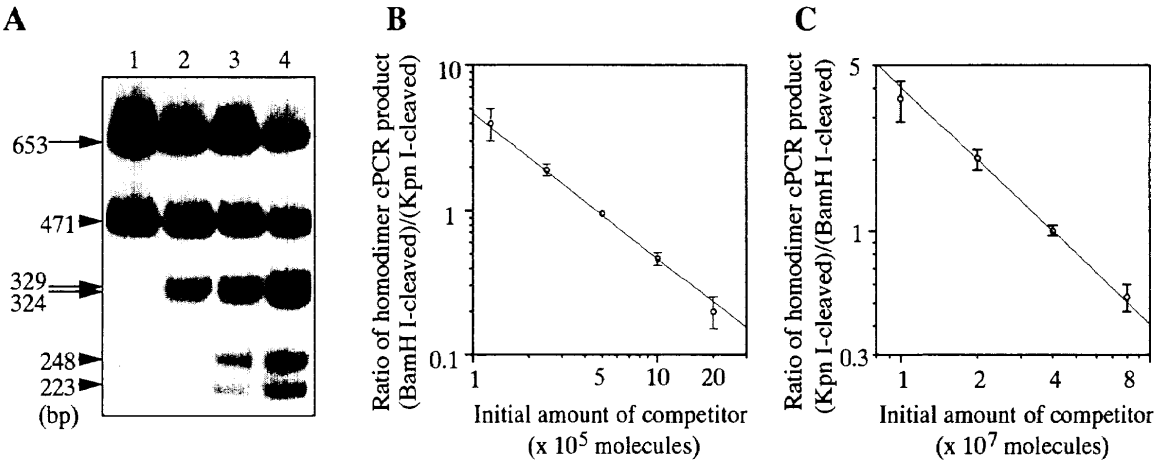

Fig. 3. Estimation of the amount of mPRL-R and mBA cDNA by cPCR in isolated pancreatic islets. An islet cDNA sample was amplified by cPCR for mPRL-R and mBA with various amounts of the competitor. (**A**) The c PCR product of islet cDNA with the competitor for mPRLR and mBA (10^5 and 10^7 molecules, respectively) was resolved by polyacrylamide gel electrophoresis after digestion with no enzyme (lane 1), *Bam*H I (lane 2), *Kpn* I (lane 3) and both enzymes (lane 4). PCR generated 653 bp mPRL-R and 471 bp mBA DNA fragments, and the products were cleaved by restriction enzymes into two pairs of fragments (329 bp and 324 bp, and 248 bp and 223 bp, respectively). (**B**) Relationship between the initial amount of the competitor for mPRL-R and the ratio of PCR product of mPRL-R cleaved by *Bam*H I to that cleaved by *Kpn* I. The latter was proportional to the square of the former. The amount of mPRL-R cDNA in islet cDNA sample was estimated to be about 1×10^6 molecules. (The competitor applied was double-strand DNA.) Vertical bars indicate S.E. (n = 3-4). (**C**) Relationship between the initial amount of the competitor for mBA and the ratio of PCR product of mBA cleaved by *Kpn* I to that cleaved by *Bam*H I. The amount of mBA cDNA in this sample was estimated to be about 8×10^7 molecules.

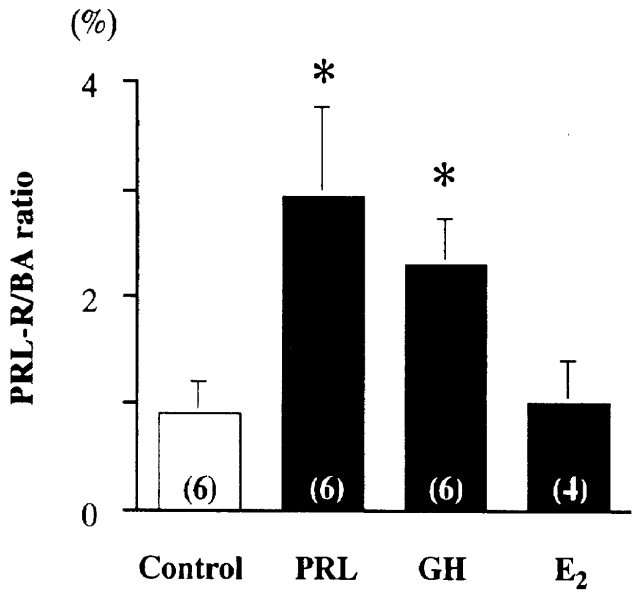

Fig. 4. Effects of hormones on expression of PRL-R mRNA in pancreatic islets in vitro. Complementary DNA samples were prepared from adult female mouse pancreatic islets cultured for 4 days with or without PRL, GH or E_2 and the ratio of mPRL-R to mBA cDNA was measured by cPCR. The ratio was significantly larger in the PRL- or GH-treated groups as compared with the control group without hormones (*P<0.05). Vertical bars indicate S.E. (n = 4-6).

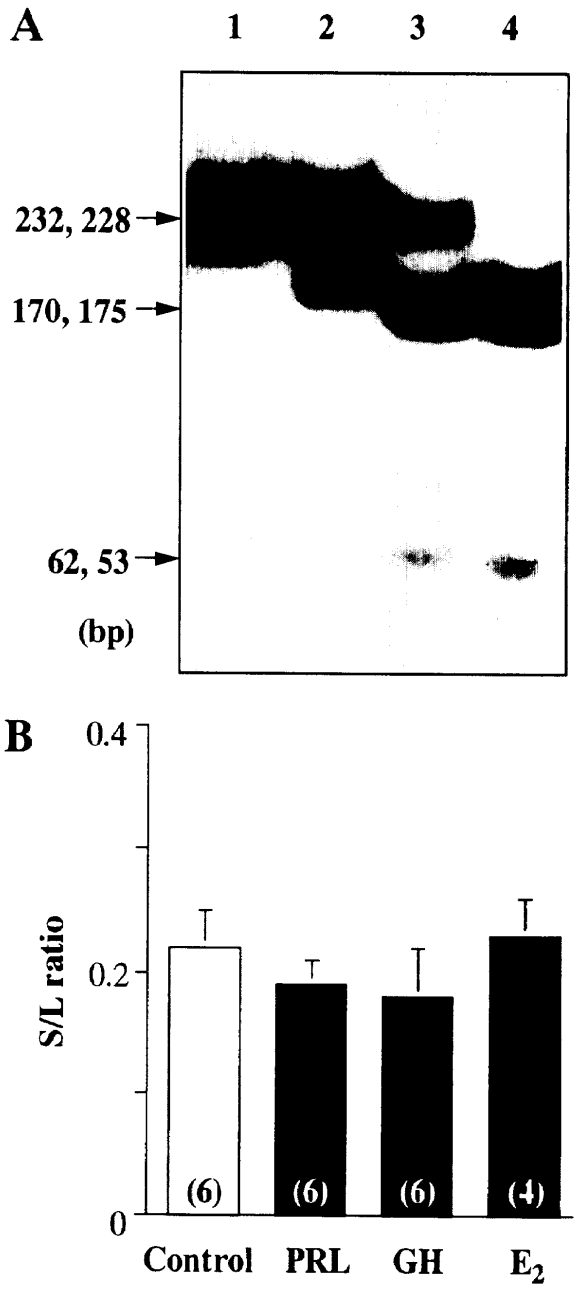

Fig. 5. Effects of hormones on the S/L ratio of mPRL-R mRNA in pancreatic islets *in vitro*. (**A**) Products of one-sided cPCR for two forms of mPRL-R cDNAs from pancreatic islets isolated from adult female mice were resolved on a 10% polyacrylamide gel after digestion with no enzyme (lane 1), *Msp* I (lane 2), *Bam*H I (lane 3), and both enzymes (lane 4). PCR generated 232-bp and 228-bp fragments of the long and short forms of mPRL-R, respectively, and the products were cleaved by restriction enzymes into two pairs of fragments (170 bp and 62 bp by *Msp* I, and 175 bp and 53 bp by *Bam*H I, respectively). (**B**) S/L ratio was measured in the cDNA sample from pancreatic islets cultured for 4 days with or without PRL, GH or E_2. There was no significant (P<0.05) difference in the S/L ratio between the groups. Vertical bars indicate S.E. (n = 4-6).

increase in pancreatic GH-R and PRL-R (Møldrup *et al.*, 1993). Members of GH/PRL family stimulate the proliferation of pancreatic B-cells and insulin secretion (Nielsen, 1982; Brelje *et al.*, 1993; Sorenson *et al.*, 1987; Brelje *et al.*, 1989). Some of these effects of GH/PRL family were confirmed in our previous study (Matsuda *et al.*, 1994) and in this study. However, the relationship between circulating level of GH/PRL family and their receptor in pancreatic islets is not well understood, although Møldrup *et al.* (1993) and Asfari *et al.* (1995) showed that PRL and GH increase both PRL-R and GH-R mRNA levels in neonatal rat pancreatic islets or in rat insulinoma cells. Our results using islets cultured from adult female mice represent more direct evidence of the role of GH and PRL on the regulation of PRL-R during pregnancy and lactation. An increase in the amount of mRNA for the long form PRL-R may be followed by an increase in the functional PRL-R protein, which in turn may lead to sensitization of the islet cells to PRL and PL. This auto-sensitization by PRL may contributes to an amplification of the PRL signal which eventually leads to the stimulation of insulin secretion in the pancreatic islets.

In rodents there exist at least two types of PRL-R, short and long forms, which are derived from alternative splicing of a precursor RNA molecule. They have the same hormone binding extracellular domain, but differ in the length and sequences of their intracellular domains which are responsible for intracellular signal transduction. Activation of PRL-dependent signaling occurs as the result of ligand-induced dimerization of receptor. Chang and Clevenger (1996) suggested that the homodimer of the long form is functional, whereas either the homodimer of the short form or the heterodimer of the two forms are unfunctional. Thus, a small change in the proportion of PRL-R isoforms could cause a big change in the PRL effects. This raises the possibility that tissue

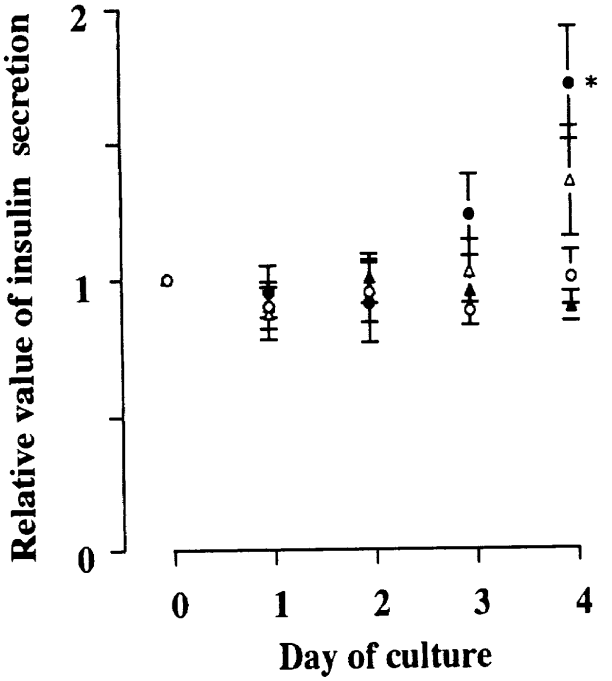

Fig. 6. Effects of oPRL, bGH, or E_2 on insulin secretion from cultured adult female mouse pancreatic islets. The graph gives the 24-hr secretion values of insulin in oPRL-treated (●), bGH-treated (△), E_2-treated (▲) and control (○) groups. The data are expressed as the amount of insulin relative to that secreted during 24-hr preculture period (Day 0). There is a significant difference in the value between the oPRL-treated and the control groups at Day 4 (*P<0.05). Vertical bars indicate S.E. (n = 6).

sensitivity to PRL could be modified by changing the proportion of each isoform. In fact, the S/L ratio of PRL-R mRNA is altered by the hormonal environment in the mouse liver (Matsuda and Mori, 1996). Hence, in this study, we examined the S/L ratio as well as the amount of extracellular region of PRL-R mRNA in pancreatic islets. One long form and three short forms of PRL-R mRNA have been found in mice tissues, whereas one long and one short form have been found in rats. We focused on the long form and a short form which is a homologue of rat short form PRL-R mRNA and the most highly-expressed form among the three short forms in murine tissues (Buck *et al.*, 1992). The results suggested that PRL-R in pancreatic islets was long form-dominant as observed in other extrahepatic tissues, and that the ratio of the two forms was not affected by PRL, GH and E_2 in the cultured pancreatic islets. Further studies on the remaining two short forms of PRL-R in mice are needed to be conclusive.

Since Becker-Andre and Hahlbrock (1989) and Gilliand *et al.* (1990) first quantified mRNA and DNA by cPCR, this procedure has been used to determine small amounts of mRNA or DNA, including rat PRL-R. In this study, PRL-R cDNA bearing two point mutations that inserted a *Kpn* I site instead of a *Bam*H I site was used as the competitor to quantify the target PRL-R cDNA, because a small difference in amplification efficiency sometime causes a large difference in the amount of PCR product. However, a competitor that is only slightly different from the target molecule dimerizes with it. There are two approaches to control the heterodimerization. One is to stop PCR during the exponentially amplifying phase when there are very few heterodimers. The other is to renature the PCR products after denaturation for random annealing. The former requires a rather sensitive method to measure the amount of the PCR products, while, in the latter, the range of target molecules that can be measured by a certain amount of competitor is rather small.

The exact amount of mPRL-R or mBA cDNA was determined by means of cPCR. However, the ratio of mPRL-R to mBA in the cDNA sample may not exactly correspond to that in the mRNA sample, because of the possible difference in the efficiency of RT between the two mRNAs. The actual ratio in the mRNA sample may be a little bigger than the ratio in cDNA, since the RT efficiency of mPRL-R may be worse than that of mBA because the distance from the poly A region, where RT starts, to the region amplified by PCR, is far. To measure the ratio of mPRL-R to mBA mRNA more precisely, the amount of mPRL-R and mBA mRNA should be estimated by using RNA competitors. In case of two mPRL-R mRNAs, there might be some differences in the efficiency of RT between them, too. The actual S/L ratio in RNA sample may be smaller than that in cDNA sample. There is heterogeneity even in each form of mPRL-R mRNA (Davis and Linzer, 1989). These mRNA species may be different from each other in the size of 3' noncoding region and in the RT efficiency. Further studies such as Northern blot analysis are needed to be conclusive.

We dealt with too small a tissue to measure its mass or total RNA content. Hence, in this study, mBA was used as a control gene by which not only to standardize the efficiencies of RNA extraction and RT but also to measure the amount of tissues. PRL and GH stimulate the proliferation of pancreatic B-cells (Nielsen, 1982; Brelje *et al.*, 1993; Matsuda *et al.*, 1994), and the amount of mRNA for cytoskeletal proteins such as mBA may increase during cell proliferation. Thus, the increase in the actual amount of mPRL-R mRNA in the tissue by PRL and GH might be more drastic than that shown in Fig. 4, although the difference might be slight because only a small part of the cells would participate in the proliferation.

In conclusion, the stimulatory effects of PRL and GH on the expression of PRL-R mRNA were demonstrated in pancreatic islets from adult female mice *in vitro* by using a quantitative cPCR method for mPRL-R and mBA cDNA. Increases in the circulating levels of the GH/PRL family may play a role in sensitizing the islet cells to PRL or PL and stimulate cell proliferation and insulin secretion in the islets during pregnancy and lactation.

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