Korean J Biol Sci 3: 311-317, 1999

Presence of Pituitary Specific Transcription Factor Pit-1 in the Rat Brain: Intracerebroventricular Administration of Antisense Pit-1 Oligodeoxynucleotide Decreases Brain Prolactin mRNA Level

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Key Words:
Pit-1
Prolactin
Rat brain
Antisense Pit-1

Prolactin (PRL) was reported to be locally synthesized in many brain areas including the hypothalamus, thalamus (TH) and hippocampus (HIP). In the pituitary lactotrophs, PRL synthesis is dependent upon a pituitary-specific transcription factor, Pit-1. In the present study, we attempted to identify Pit-1 or Pit-1-like protein in brain areas known as the synthetic sites of PRL. Reverse transcription-polymerase chain reaction (RT-PCR) and Northern blot analysis showed the same Pit-1 transcripts in brain areas such as the medial basal hypothalamus (MBH), preoptic area (POA), TH, and HIP with the Pit-1 transcripts in the anterior pituitary (AP). Electrophoretic mobility shift assay (EMSA) was run with nuclear protein extracts from brain tissues using a double strand oligomer probe containing a putative Pit-1 binding domain. Shifted bands were found in EMSA results with nuclear proteins from MBH, POA, TH and HIP. Specific binding of the Pit-1-like protein was further confirmed by competition with an unlabeled cold probe. Antisense Pit-1 oligodeoxynucleotide (Pit-1 ODN), which was designed to bind to the Pit-1 translation initiation site and block Pit-1 biosynthesis, was used to test Pit-1 dependent brain PRL transcription. Two nmol of Pit-1 ODN was introduced into the lateral ventricle of a 60-day old male rat brain. RNA blot hybridization and in situ hybridization indicated a decrease of PRL mRNA signals by the treatment of Pit-1 ODN. Taken together, the present study suggests that Pit-1 may play an important role in the transcriptional regulation of local PRL synthesis in the brain.

Prolactin (PRL) was originally identified as an anterior pituitary (AP) hormone playing a regulatory role in mammalian milk synthesis. Previous studies indicated that PRL has various target organs and tissues and functional varieties (reviewed by Ben-Jonathan et al., 1996). PRL regulates many physiological processes including the regulation of mammary gland development, initiation and maintenance of lactation, immune modulation, osmo-regulation, and behavioral modification. Recently, almost all of the PRL targets including the brain were known to synthesize their own PRL by themselves (Ben-Jonathan et al., 1996). Locally synthesized PRL exerts many kinds of cellular functions such as mitogenic, morphogenic or secretory activities in their synthetic sites and/or vicinity.

The pituitary-specific expression of PRL and growth hormone (GH) is due to the 5′ flanking region containing binding sites for a transcription factor, Pit-1 or GHF-1 (Ingraham et al., 1990; Karin et al., 1990). Pit-1 was originally identified to regulate fetal pituitary development as a pattern formation gene (Ingraham et al., 1988, 1990; Karin et al., 1990; Li et al., 1990). Pit-1 is a member of the superfamily of DNA binding proteins containing a homeodomain (Bodner et al., 1988; Ingraham et al., 1988, 1990). Pit-1 is closely related to the Oct-1, Oct-2 and Unc-86 proteins, which contain a second highly conserved sequence motif known as the POU-specific domain (Herr et al., 1988). Pit-1 binds 2 and 8 sites in the 5′-flanking regions of GH and PRL genes, respectively (Lefevre et al., 1987; Nelson et al., 1988). Pit-1 also binds to its own promoter region and autoregulates the transcription of the Pit-1 gene by itself (Chen et al., 1990). Recently, it was shown that Pit-1 is synthesized in several tissues including human and rat placentas, hemopoietic and lymphoid tissues producing a PRL or PRL-like protein, and plays a transactivational role for local PRL (or PRL-like protein) synthesis (Delhase et al., 1993; Bamberger et al., 1995; Lee et al., 1996; Lee et al., 1998).

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In the present study, we presumed that Pit-1 is locally synthesized and trans-activates PRL gene expression in the brain. We attempted to clarify this hypothesis using Northern blot hybridization, electrophoretic mobility shift assay (EMSA) and targeted disruption of Pit-1 synthesis with intracerebroventricular (ICV) administration of the antisense Pit-1 oligodeoxynucleotide.

Materials and Methods

Animal and tissue preparations

Adult male Sprague-Dawley rats were used in the present experiments. Animals were housed in the normal light dark cycle (light on 0600 h) and water and food were fully supplied. Tissue samples including brain areas such as the medial basal hypothalamus (MBH), preoptic area (POA), thalamus (TH) and hippocampus (HIP) were collected from animals. For in situ hybridization analysis, animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.2) through the left cardiac ventricle.

PCR cloning of PRL and Pit-1 cDNAs

Partial cDNAs of Pit-1 and PRL were cloned with RT-PCR using primer sets based on the known sequences (Cooke et al., 1980; Ingraham et al., 1990; Karin et al., 1990; Wilson et al., 1992). The PCR-cloned Pit-1 and PRL cDNA fragments were used as templates for cRNA probes for Pit-1 mRNA determination using Northern blot hybridization and PRL mRNA using in situ hybridization histochemistry, respectively. The PCR-cloned PRL cDNA was also used as the cDNA probe for the slot blot hybridization of brain PRL mRNA. For amplification of PRL cDNA, upstream primer (5′-AAA TGT GCA GAC CCT GCC AGT C-3′) and downstream primer (5′-CAT CAG GAG CTT CAT GGA TTC C-3′) were synthesized based on previous reports (Cooke et al., 1980; Wilson et al., 1992). For PCR of Pit-1, upstream primer (5′-CAA CCT TTC ACC TCG GCT GAT AC-3′) and downstream primer (5′-GGG TGT GGT CTG GAA ACT TGT AAA G-3′) were designed based on previous reports (Ingram et al., 1990; Karin et al., 1990). Amplification products were inserted into pGEM-T easy vector (Promega) and sequenced using an automatic DNA sequencer (Korea Basic Science Institute).

Northern blot and slot blot hybridization

To determine the levels of brain Pit-1 and PRL mRNA, Northern blot and slot blot hybridization were employed, respectively. Total cytoplasmic RNA was extracted using the guanidinium thiocyanate-acid phenol-chloroform method (Chomczyski and Sacchi, 1987). RNA samples (20 μg) were separated on a 1.2% agarose-formaldehyde gel, and transferred onto a positively charged N^+ nylon membrane (Amersham). To determine the level of PRL mRNA, RNA samples (5 μg) were blotted onto a nylon membrane with a slot blot kit (Hoeffer). Membranes were hybridized with a ^{32}P-labeled Pit-1 cRNA probe or a ^{32}P-labeled PRL cDNA probe in hybridization solution with a specific activity of 1×10^6 cpm/ml, respectively. Final post-hybridization washes were in 0.1 × SSC at 60℃. Autoradiographic images of X-ray film were normalized with densities of ribosomal RNA bands and calculated as a percent of control values.

Probes

Complementary DNA probe for slot blot hybridization of PRL mRNA was labeled with ^{32}P-dCTP (Amersham) using a random primer labeling kit (Boehringer Mannheim) with EcoRI digested PCR-cloned PRL cDNA. RNA probes were used for Northern blot hybridization of Pit-1 mRNA and in situ hybridization of PRL mRNA. After linearization with NcoI, ^{32}P- or ^{35}S-labeled cRNA probes were synthesized using SP6 RNA polymerase. Radiolabeled probes were separated from the free isotope with a Nick column (Pharmacia).

Electrophoretic Mobility Shift Assay (EMSA)

Nuclear protein extracts from different rat tissues known as synthetic sites for PRL were prepared according to the method of Andrews and Faller (1991), utilizing the cocktail of protease inhibitors. A double-stranded oligodeoxynucleotide probe containing the sequence of the proximal auto-feedback Pit-1 binding site in the Pit-1 promoter (5′-GCC GCC CTG ATG TAT ATA TGC AAT AGG GAG C-3′) was synthesized as a complementary pair on an automatic DNA synthesizer as described previously (Chen et al., 1990). The probe was end-labeled with ^{32}P-γ-ATP in a reaction catalyzed by DNA polynucleotide kinase and purified over a Nick column before use. The binding assay was performed as described (Andrews and Faller, 1991) using nuclear proteins (2 μg from AP and 5 μg from other tissues, respectively), 20,000 cpm of probe and 1 μg of poly (dI-dC) in a buffer containing 10 mM Tris (pH 7.5, 50 mM NaCl, 1 mM EDTA, 5 mM $MgCl_2$, and 5% glycerol). Upon completion of the reaction (performed in a 20 μl volume, for 20 min at room temperature), the protein/DNA complexes were separated by electrophoresis on a 4% nondenaturing polyacrylamide gel using a running buffer containing 0.05 M Tris, 0.38 M glycine, and 2 mM EDTA, pH 8.8. The gels were then dried and exposed to film at -85℃.

Targeted disruption of Pit-1 synthesis

An antisense oligodeoxynucleotide (Pit-1 ODN) directed against the sequence surrounding the first ATG codon (Ingraham et al., 1990) in the rat Pit-1 gene (5′-TTG GCA ACT CAT TCC CAC AAG-3′) was used to disrupt Pit-1 synthesis. An ODN containing the same nucleotide composition of the antisense ODN, but in scrambled order (Pit-1 SCR: 5′-TCG CCA ATA GCC TAC TTA GAC-3′) was used as a control. This scrambeled sequence does not bear similarity with any sequence

deposited thus far in the NCBI GenBank. To determine the selective effect of ODN on Pit-1 synthesis, we determined the level of the Pit-1 protein using EMSA.

Intracerebroventricular (ICV) injection of antisense Pit-1 oligodeoxynucleotide

For the ICV injection, the ODNs were diluted in saline at a final concentration of 0.5 mM. Under pentobarbital (7.5 mg/kg BW) and ketamine hydrochloride (25 mg/kg BW) anesthesia, a polyethylene guide cannula (od 1.05 mm, id 0.35 mm) with the inner stylet (27 gauge) was stereotaxically implanted into a lateral ventricle (1.2 mm rostral to the bregma, 1.2 mm lateral from the midline, and 4.5 mm vertical from the surface of the skull) of 60-day old male SD rats and fixed in place with anchor screws and dental cement. After a recovery period of 1 week, the inner stylet was removed and then ODNs (2 nmol) were ICV injected with a Hamilton syringe once a day for 2 consecutive days. The amount of ODN injected was reported to be more than sufficient to block the synthesis of the target protein (Ogawa et al., 1994; Seong et al., 1998).

To determine the functional consequences of Pit-1 ODN-induced inhibition of Pit-1 synthesis, we sought to determine the level of PRL mRNA using slot blot hybridization 2 days after ICV injection.

In situ hybridization

The procedure employed is that described previously (Simmons et al., 1989) with minor modifications. The brains were fixed by transcardiac perfusion with 4% paraformaldehyde in phosphate buffered saline (pH 7.4), followed by overnight post-fixation in the same fixative containing 10% sucrose. The tissues were frozen on dry ice and stored at -80℃ until sectioning. Sections with 20 μm thickness were obtained using a sliding microtome, mounted onto ProbeOn™ Plus microscope slides (Fisher Scientific), and dried under vacuum overnight before hybridization. The sections were overlaid with 70 μl of hybridization solution containing 5×10^6 cpm of probe per ml and hybridized overnight at 60℃. Post hybridization washes were carried out as recommended (Simmons et al., 1989).

Statistical analysis

To determine the statistical significance of Pit-1 ODN on the mRNA level of brain PRL, we applied student T-tests between Pit-1 SCR and Pit-1 ODN injected groups. The significance was determined at the level of $p<0.05$.

Results

Northern blot analysis of Pit-1 mRNA

Pit-1 mRNA was determined in the brain regions by Northern blot hybridization using ^{32}P-labeled cRNA probe. RT-PCR was performed to make a cDNA fragment of Pit-1 from RNA extracted from hypothalamic tissues. The Pit-1 cDNA fragment in the brain had exactly the same sequence with the known sequence of Pit-1 cDNA in AP (data not shown).

In agreement with previous reports in AP (Ingraham et al., 1988; Day and Day, 1994), major and minor transcripts of approximately 2.5 kb and 1.2 kb were detected in MBH, POA, TH and HIP of the brain as well as AP (Fig. 1). Levels of Pit-1 mRNA in the brain tissues were relatively low compared to the level of Pit-1 mRNA in AP when the same amount of RNA (20 μg) was loaded on each lane.

Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assay (EMSA) was carried out to determine the binding protein to a putative Pit-1 binding domain in the promoter region of the Pit-1 gene. Nuclear protein fractions from rat tissues including MBH, POA, TH, and HIP were incubated with a double strand oligomer probe. A single shifted band of the same size with a positive control tissue of AP was found in MBH, POA, TH, HIP, mammary gland and placenta (Fig. 2). Therefore, a putative Pit-1 (or Pit-1-like protein) in these tissues seemed to bind the Pit-1 binding domain of an oligomer probe. On the contrary, nuclear proteins from the thymus, spleen, and uterus did not generate a specific shifted band as found in AP, suggesting that these tissues do not synthesize Pit-1 (or Pit-1 like protein) with the binding activity to the Pit-1 binding domain. Different forms of shifted bands were shown in the cerebellum and decidua, suggesting that different forms of Pit-1 or different protein sharing binding activity with Pit-1 may be synthesized in these tissues.

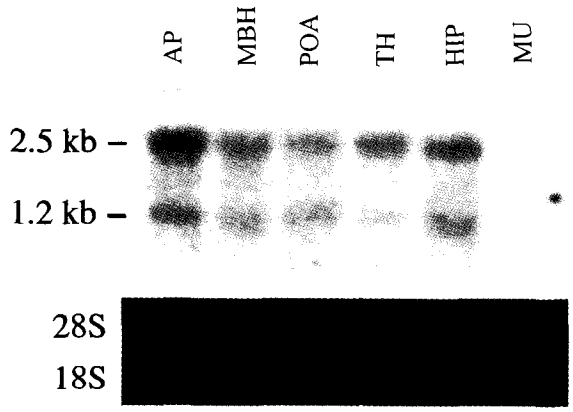

Fig. 1. Northern blot analysis of Pit-1 mRNA in the rat brain. RNA samples (20 μg) were run on 1.2% agarose gel, transferred onto nylon membrane, and hybridized with a ^{32}P-labeled Pit-1 cRNA probe. The upper panel represents autoradiography of Pit-1 mRNA bands showing a 2.5 kb major transcript and 1.2 kb minor transcript in the positive control tissue of the anterior pituitary (AP), medial basal hypothalamus (MBH), preoptic area (POA), thalamus (TH), and hippocampus (HIP). No positive signal was observed in the muscle (MU). Lower panel shows 28 S and 18 S ribosomal RNA bands.

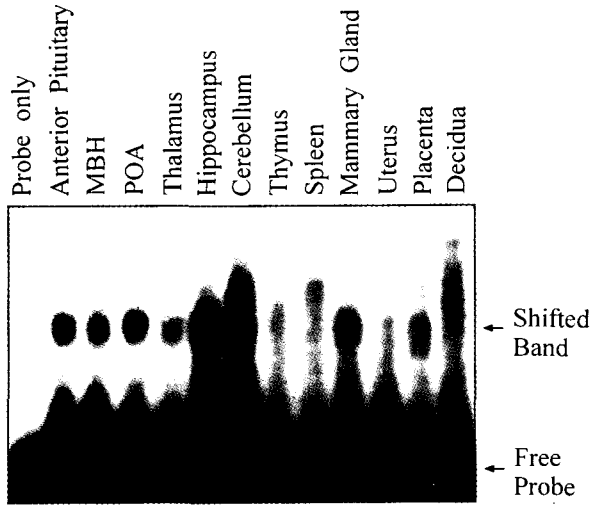

Fig. 2. Electrophoretic mobility shift assay (EMSA) of Pit-1-like proteins in various tissues and organs including the medial basal hypothalamus, a preoptic area in the brain. Nuclear protein extracts (2 μg for anterior pituitary and 5 μg for other tissues) were incubated with ^{32}P-labeled double strand oligomer probe containing a Pit-1 binding domain and poly (dI-dC). Shifted bands were separated from the free probe with a 4% nondenaturing polyacrylamide gel.

We focused on the specific shifted band of Pit-1 found in brain tissues only, and further confirmed the presence of the Pit-1 protein in brain tissues using EMSA as a competitor. ^{32}P-labeled probe competed with excess amounts of unlabeled cold probe to bind the nuclear protein fractions that have binding activity to the Pit-1 binding domain. Shifted bands found in the HIP, MBH, POA and TH disappeared by addition of excess amounts of cold probes (Fig. 3).

Effect of ICV injection of antisense Pit-1 oligodeoxynucleotide (ODN)

To target the synthesis of Pit-1 in the brain, we injected antisense Pit-1 oligodeoxynucleotide (Pit-1 ODN) into the lateral ventricle of the male rat. To validate the

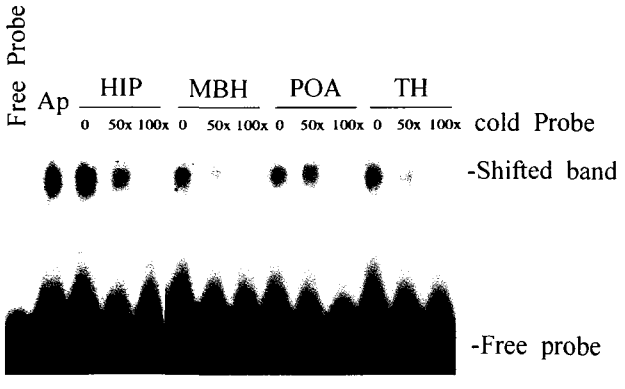

Fig. 3. Competition of electrophoretic mobility shift assay. Excess amounts of unlabeled probes (50x and 100x) were added in the reaction mixture to compete with the radiolabeled probe. Nuclear protein fractions were extracted from the hippocampus (HIP), medial basal hypothalamus (MBH), preoptic area (POA) and thalamus (TH). AP, anterior pituitary.

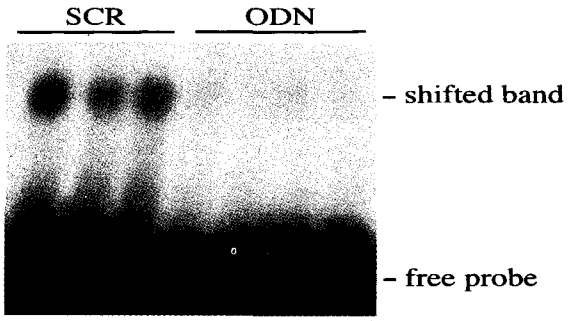

Fig. 4. Validation of the selective effect of antisense Pit-1 oligodeoxynucleotide (ODN) on the biosynthesis of Pit-1. Pit-1 ODN and SCR (2 nmol each) were ICV injected into the lateral ventricle twice, and 2 days later nuclear proteins were extracted from the hypothalami. Nuclear extracts (5 μg) were analysed with EMSA. Pit-1 ODN clearly removed the shifted bands of the binding protein with the Pit-1 specific oligomer probe.

selective effect of antisense ODN on Pit-1 protein synthesis, EMSA was carried out to determine the binding activity of Pit-1 protein from the hypothalamic nuclear extracts. As shown in Fig. 4, shifted bands were almost completely removed in the Pit-1 ODN injected rat hypothalami. To determine the inhibitory effect of Pit-1 synthesis on PRL mRNA levels, we measured PRL mRNA levels, using slot blot hybridization, in brain tissues injected with Pit-1 ODN. Pit-1 ODN significantly suppressed PRL mRNA levels in the brain tis-

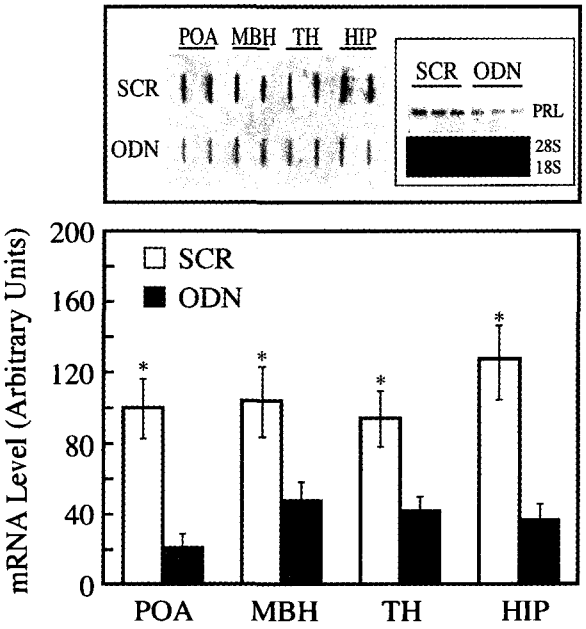

Fig. 5. Effect of antisense Pit-1 ODN on the PRL mRNA level. Two nmol of antisense Pit-1 ODN or scramble (SCR) were injected into the lateral ventricle twice, and RNA samples were purified from the preoptic area (POA), medial basal hypothalamus (MBH), thalamus (TH), and hippocampus (HIP). Slot blot hybridization (using 5 μg RNA samples) was performed to determine the level of PRL mRNA with a ^{32}P-labeled PRL cDNA probe. Upper panel shows the representative autoradiography and lower panel shows the mean (±SEM) of three repeated experiments. Inlet of upper panel shows the effect of antisense Pit-1 ODN on prolactin (PRL) mRNA in the anterior pituitary.
* Significantly different at the level of $p<0.05$.

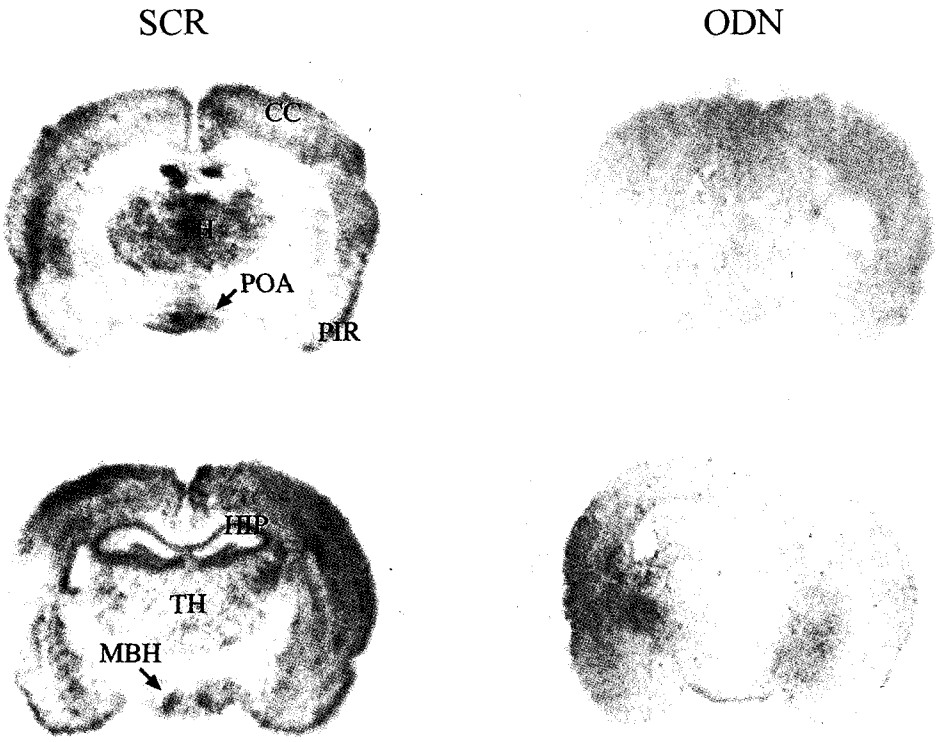

Fig. 6. In situ hybridization analysis showing the effect of antisense Pit-1 ODN on the brain PRL mRNA. Bright fields of autoradiography (10x magnification) represent PRL mRNA signals in brain areas including the hippocampus (HIP), thalamus (TH), preoptic area (POA), and medial basal hypothalamus (MBH) in the Pit-1 SCR injected control rat brain (left panels). Antisense Pit-1 ODN almost completely removed the positive signals of PRL mRNA (right). Upper panels show the rostral sections of lower panels. CC, cerebral cortex; PIR, piriform cortex.

sues such as the POA, MBH, TH and HIP as well as the PRL mRNA level in AP (Fig. 5).

The effect of Pit-1 ODN on the mRNA level of brain PRL was further confirmed with in situ hybridization histochemistry using a ^{35}S-labeled PRL cRNA probe (Fig. 6). PRL mRNA signals were found in many brain regions including the HIP, TH, POA and MBH of the SCR injected control animal. The PRL mRNA signals nearly disappeared with ICV injection of Pit-1 ODN. This result differed from the result of slot blot analysis showing a 60-80% reduction in the PRL mRNA level by Pit-1 ODN. This discrepancy may be due to different determination methods and sensitivities between the two techniques. It is presumed that the present in situ hybridization analysis did not generate specific signals from reduced PRL mRNA levels by Pit-1 ODN shown in the slot blot analysis.

Discussion

PRL affects many physiological processes in various targets such as the mammary gland, placenta, uterus and brain. Furthermore, PRL is involved in many kinds of cellular functions such as mitogenic, morphogenic or secretory activities and osmo-regulation. Recently, PRL was reported to be locally synthesized in several brain areas including the hypothalamus, TH and HIP (Ben-Jonathan et al., 1996).

PRL gene transcription in AP is dependent upon trans-activation by a pituitary specific transcription factor, Pit-1, by binding to eight binding motifs in the PRL gene promoter region (Nelson et al., 1988). Moreover, Pit-1 was reported to play an important role in the trans-activation of PRL or PRL-like protein synthesized in other tissues such as the placenta and lymphoid tissues (Delhase et al., 1993; Bamberger et al., 1995; Lee et al., 1996; Lee et al., 1998). Therefore, we hypothesized that Pit-1 may play a role in the biosynthesis of brain PRL.

We report here that Pit-1 is locally synthesized in the rat brain region and regulates brain PRL gene expression, based on the electrophoretic mobility shift assay, PCR, and Northern blot analysis. Data from the electrophoretic mobility shift assay and Northern blot hybridization showed that the Pit-1 protein as well as mRNA is present in brain regions such as the hypothalamus, TH, and HIP. According to the present Northern blot analysis, the same major and minor transcripts were found to be transcribed from the Pit-1 gene in the brain as in AP, though the level of brain Pit-1 mRNA was relatively low compared to the level of AP Pit-1 mRNA. Therefore, the same Pit-1 found in AP may play a role in the regulation of PRL gene transcription in the brain.

Recently, several results suggested that Pit-1 is locally synthesized and plays a role in the regulation of local GH and PRL family genes in the placenta, hemopoietic and lymphoid tissues as described above. In addition, phylogenetically conserved Pit-1 was found in the salmon pituitary and it was believed to regulate the expression of salmon PRL (Elsholtz et al., 1992; Ono et al., 1994). Taken together with the previous data, the present results highly suggest that Pit-1 may play a role as a target gene-specific transcription factor in whichever tissues it is found.

To test this hypothesis, we determined the level of brain PRL mRNA after ICV injection of antisense Pit-1 ODN. Pit-1 ODN markedly suppressed the level of brain PRL mRNA as well as PRL mRNA in AP. The present result suggests that Pit-1 ODN exerts its effect on the transcription of the PRL gene through the inhibition of Pit-1 biosynthesis and, in turn, the low level of Pit-1 may not sufficiently trans-activate PRL gene expression.

It is so far unclear how the ODN can reach cells in deep areas of the brain and play an inhibitory role in protein synthesis. ODN may be diffused through the periventricular wall into brain areas including the hypothalamus, HIP and TH as previously suggested (Pellegrini et al., 1996; Cui et al., 1999). Cui et al. (1999) showed that ICV injected biotinylated-ODN reached even to cerebral and cerebellar layers within 24 hr, and the level of ODN was maintained up to 2 days after infusion. Although the precise pathway of ODN into the cell is still unclear, the perinuclear localization of ODN was observed in several studies (Agarawal et al., 1992; Whitesell et al., 1993; Cui et al., 1999). In part, endocytosis may be one mechanism through which cells uptake charged ODN (Yakubov et al., 1989). Previous reports suggest that antisense ODN may block the translation of the protein by binding to the translation initiation site of mRNA or DNA/RNA double strand may facilitate the RNase H digestion of mRNA (Landgraf, 1996; Roush, 1997).

The decrease in PRL mRNA level of AP may be due to the partial delivery of Pit-1 ODN to AP through the hypothalamo-hypophysial portal system because median eminence, the hypothalamic end of the portal vein, lies outside the blood-brain barrier (Pellegrini et al., 1996). It may also be due to an indirect mechanism through the effect of Pit-1 ODN on the PRL release inhibiting factor or activating factor in the hypothalamus although it has not been tested yet.

In the present study, we found the Pit-1 transcript corresponding to the major splice variant of the α form using RT-PCR and Northern blot analysis. However, we could not find any other minor splice variants such as β and τ forms as suggested by previous studies (Day and Day, 1994; Konzak and Moore, 1992; Voss et al., 1993). β and τ forms of splice variants may not be present at all or be much less than the α form in the brain. Pit-1 β was known to trans-activate the PRL and GH gene promoter and be a more potent trans-activator for the GH gene promoter than Pit-1 α (Konzak and Moore, 1992). Further studies are needed to clarify the presence of Pit-1 β and τ, and their possible role in the brain.

Pit-1 has been known to be essential in the trans-activation of placental lactogen (PL) genes and/or GH variant genes in the rat and human placenta (Bamberger et al., 1995; Lee et al., 1996; Lee et al., 1998). On the other hand, the placenta specific expression of PLs was reported to be due to other regulators such as GATA factors for the trans-activation of the mouse PL-I gene (Ng et al., 1994), and transcriptional enhancer factor-1 (TEF-1) for human chorionic somatomammotropin (Lytras and Cattini, 1994). Recently, the PRL regulatory element binding protein (PREB) was identified and characterized to bind to the PRL promoter in combination with Pit-1 in the rat pituitary (Fliss et al., 1999). PREB mRNA was also found in the brain and other tissues known as RRL biosynthetic sites. Therefore, we could not exclude the possibility that PREB and Pit-1 may coordinately work to regulate the transcription of the PRL gene in the brain.

Acknowledgements

This study was supported by a grant from the Korea Research Foundation (1997-001-D00354) and partially supported by a grant from the Korea Science and Engineering Foundation through HRC (1998G0103). We thank the Korea Basic Science Institute for sequencing cDNA fragments of PRL and Pit-1.

References

Agarawal S, Sarin PS, Zamecnik M, Zamecnik PC (1992) Ceullular uptake and anti-HIV activity of oligonucleotide and therir analogs. In: Erickson RP and des Izant JG (eds), Gene Regulation: Biology of Antisense RNA and DNA, Raven Press, New York, pp 173-228.

Andrews NC and Faller DV (1991) A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res* 19: 2499-1504.

Bamberger AM, Bamberger CM, Pu LP, Puy LA, Loh YP, and Asa SL (1995) Expression of Pit-1 messenger ribonucleic acid and protein in the human placenta. *Clin Endocrinol Metab* 80: 2021-2026.

Ben-Jonathan N, Mershon JL, Allen DL, and Steinmetz RW (1996) Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr Rev* 17: 639-669.

Bodner M, Castrillo JL, Theill LE, Deerinck T, Ellisman M, and Karin M (1988) The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. *Cell* 55: 505-518.

Chen R, Ingraham HA, Treacy MN, Albert VR, Wilson L, and Rosenfeld MG (1990) Autoregulation of Pit-1 gene expression mediated by two cis-active promoter elements. *Nature* 346: 583-586.

Chomczynski P, and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Ann Biochem* 162: 156-159.

Cooke NE, Coit D, Weiner RI, Baxter JD, and Martial JA (1980) Structure of cloned DNA complementary to rat prolactin messenger RNA. *J Biol Chem* 255: 6502-6510.

Cui J-K, Hsu CY, and Liu PK (1999) Suppression of postischemic hippocampal nerve growth factor expression by a

c-fos antisense oligodeoxynucleotide. *J Neurosci* 19: 1335-1344.

Day RN, and Day KH (1994) Specific repression of rat prolactin gene expression in transplanted tumor cells. *Mol Endocrinol* 8: 12-20.

Delhase M, Vergani P, Malur A, Hooghe-Peters EL, and Hooghe RJ (1993) The transcription factor Pit-1/GHF-1 is expressed in hemopoietic and lymphoid tissues. *Eur J Immunol* 23: 951-955.

Elsholtz HP, Majumdar-Sonnylal S, Xiong F, Gong Z, and Hew CL (1992) Phylogenetic specificity of prolactin gene expression with conservation of Pit-1 function. *Mol Endocrinol* 6: 515-522.

Fliss MS, Hinkle PM, and Bancroft C (1999) Expression cloning and characterization of PREB (prolactin regulatory element binding), a novel WD motif DNA-binding protein with a capacity to regulate prolactin promoter activity. *Mol Endocrinol* 13: 644-657.

Herr W, Sturm RA, Clerc RG, Corcoran LM, Baltimore D, Sharp PA, Ingraham HA, Rosenfeld MG, Finney M, Ruvkun G, and Horvitz R (1988) The POU domain: a large conserved region in the mammalian Pit-1, Oct-1, Oct-2, and *Caenorhabditis elegans unc-86* gene products. *Genes & Dev* 2: 1513-1516.

Ingraham HA, Chen RP, Mangalam HJ, Elsholtz HP, Flynn SE, Lin CR, Simmons DM, Swanson L, and Rosenfeld MG (1988) A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. *Cell* 55: 519-529.

Ingraham HA, Flynn SE, Voss JW, Albert VR, Kapiloff MS, Wilson L, and Rosenfeld MG (1990) The POU-specific domain of Pit-1 is essential for sequence-specific, high affinity DNA binding and DNA-dependent Pit-1-Pit-1 interactions. *Cell* 61: 1021-1033.

Karin M, Theill L, Castrillo JL, McCormick A, and Brady H (1990) Tissue-specific expression of the growth hormone gene and its control by growth hormone factor-1. *Recent Prog Horm Res* 46: 43-58.

Konzak KE, and Moore DD (1992) Functional isoforms of Pit-1 generated by alternative messenger RNA splicing. *Mol Endocrinol* 6: 241-247.

Landgraf R (1996) Antisense targeting in behavioural neuroendocrinology. *J Endocrinol* 151: 333-340.

Lee BJ, Jeong JK, Kim JH, Kang SG, Kim MO, and Choi WS (1996) Local expression of a POU family transcription factor, Pit-1, in the rat placenta. *Mol Cell Endocrinol* 118: 9-14.

Lee CK, Kang HS, Lee BJ, Kang HM, Choi WS, and Kang SG (1998) Effects of dopamine and estrogen on the regulation of *Pit-1a, Pit-1b*, and *PL-II* gene expression in the rat placenta. *Mol Cells* 8: 205-211.

Lefevre C, Imagawa M, Dana S, Grindlay J, Bodner M, and Karin M (1987) Tissue-specific expression of the human growth hormone gene is conferred in part by the binding of a specific trans-acting factor. *EMBO J* 6: 971-981.

Li S, Crenshaw EB 3rd, Rawson EJ, Simmons DM, Swanson LW, and Rosenfeld MG (1990) Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *Pit-1*. *Nature* 347: 528-533.

Lytras A, and Cattini PA (1994) Human chorionic somatotropin gene enhancer activity is dependent on the blockade of a repressor mechanism. *Mol Endocrinol* 8: 478-489.

Nelson C, Albert VR, Elsholtz HP, Lu LI, and Rosenfeld MG (1988) Activation of cell-specific expression of rat growth hormone and prolactin genes by a common transcription factor. *Science* 239: 1400-1405.

Ng Y-K, George KM, Engel JD, and Linzer DIH (1994) GATA factor activity is required for the trophoblast-specific transcriptional regulation of the mouse placental lactogen I gene. *Development* 120: 3257-3266.

Ogawa S, Olazabal UE, Parhar IS, and Pfaff DW (1994) Effects of intrahypothalamic administration of antisense DNA for progesterone receptor mRNA on reproductive behavior and progesterone receptor immunoreactivity in female rat. *J Neurosci* 14: 1766-1774.

Ono M, Harigai T, Kaneko T, Sato Y, Ihara S, and Kawauchi H (1994) Pit-1/GH factor-1 involvement in the gene expression of somatolactin. *Mol Endocrinol* 8: 109-115.

Pellegrini E, Bluet-Pajot MT, Mounier F, Bennett P, Kordon C, and Epelbaum J (1996) Central administration of a growth hormone (GH) receptor mRNA antisense increases GH pulsatility and decreases hypothalamic somatostatin expression in rats. *J Neurosci* 16: 8140-8148.

Roush W (1997) Antisense aims for a renaissance. *Science* 276: 1192-1193.

Seong JY, Kang SS, Kam K, Han Y-G, Kwon HB, Ryu K, and Kim K (1998) Differential regulation of gonadotropin-releasing hormone (GnRH) receptor expression in the posterior mediobasal hypothalamus by steroid hormones: implication of GnRH neuronal activity. *Mol Brain Res* 53: 226-235.

Simmons DM, Arriza JL, and Swanson JW (1989) A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radiolabeled single-strand RNA probes. *J Histotechnol* 12: 169-181.

Voss JW, Wilson L, Rhodes SJ, and Rosenfeld MG (1993) An alternative Pit-1 RNA splicing product reveals modular binding and nonmodular transcriptional activities of POU-specific domain. *Mol Endocrinol* 7: 1551-1560.

Whitesell L, Geselowitz D, Chavany C, Fahmy B, Walbridge S, Alger JR, and Neckers LM (1993) Stability, clearance, and disposition of intraventricularly administered oligodeoxynucleotides: implications for therapeutic application within the central nervous system. *Proc Natl Acad Sci USA* 90: 4665-4669.

Wilson DM 3rd, Emanuele NV, Jurgens JK, and Kelley MR (1992) Prolactin message in brain and pituitary of adult male rats is identical: PCR cloning and sequencing of hypothalamic prolactin cDNA from intact and hypophysectomized adult rats. *Endocrinology* 131: 2488-2490.

Yakubov LA, Deeva EA, Zarytova VF, Ivanova EM, Ryte AS, Yurchenko LV, and Vlassovm VV (1989) Mechanism of oligonucleotide uptake by cells: involvement of specific receptors? *Proc Natl Acad Sci USA* 86: 6454-6458.

[Received June 8, 1999; accepted July 5, 1999]