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Developmental Expression and Localization of IA-2 mRNA in Mouse Neuroendocrine Tissues

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Islet antigen (IA)-2 is a novel autoantigen of insulin-dependent diabetes mellitus (IDDM), and belongs to a new class within the receptor-type protein tyrosine phosphatase (PTP) family characterized by lack of PTP enzymatic activity with conventional substrates. Its expression is restricted primarily to the pancreas, pituitary, and brain with the highest level in the brain. IA-2 mRNA expressions in the brain, pituitary and pancreas of 1-, 4-, and 8-week-old mice were examined. *In situ* hybridization of the brain revealed that IA-2 mRNA was expressed in the cerebral cortex, hippocampus, thalamus, choroid plexus, hypothalamus, Purkinje cells, and granular layer of the cerebellum. In the pituitary, IA-2 mRNA was located in the anterior and posterior pituitary by *in situ* hybridization. The pattern of IA-2 mRNA expression in normal male mouse brain at 1, 4, and 8 weeks of age by the Northern blot analysis was similar to that in the pituitary by RT-PCR analysis. The expression level was higher at 4 weeks and lower at 1 week of age. In the pancreas, IA-2 mRNA expressions detected by RT-PCR were highest at 8 weeks of age. These results indicated that the amount of mRNA expression increased in accordance to development in brain, pituitary, and pancreas.

Key Words: **autoantigen; autoimmunity; development; diabetes; prediction.**

Insulin-dependent diabetes mellitus (IDDM) is caused by destruction of fl-cells in the islets of Langerhans and is a chronic autoimmune disease that induce lymphocytic infiltration of pancreatic islets and circulating serum autoantibodies to various islet-specific antigens. Early in life environmental factors (such as viral infections and drugs or toxin exposure) operating in a genetically susceptible host initiate a destructive autoimmune process. The major autoantigens in the islets have been identified; viz., glutamic acid decarboxylase (GAD), insulin, and recently discovered protein tyrosine phosphatase (PTP)-like molecule, islet antigen (IA)-2. Detection of IA-2 autoantibody can be established earlier and more specifically than other autoantibodies in the diabetic-related family-tree members many months or years before and/or at the onset of IDDM. Many diabetes-associated autoantibodies; i.e., anti-GAD and anti-IA-2 appear in the living system by 5 years of age (1). Therefore, IA-2 autoantibody may serve as a useful and reliable predictive marker of IDDM (2–6).

IA-2 cDNA was obtained from the human insulinoma subtraction library (7). IA-2 is a new member of the receptor-type PTP family and has a signal peptide, an extracellular domain with a unique cystein-rich segment, a transmembrane region, and an intracellular domain with a single PTP domain. IA-2 is expressed predominantly in the brain and pancreas (7). Comparison of the PTP catalytic domain of IA-2 with other PTP catalytic domains has revealed several substitutions in the highly conserved residues, including an aspartic acid replacement for alanine that is critical for PTP enzymatic activity (8, 9). In fact, IA-2, despite its close resemblance in sequence to other PTPs, fails to display PTP enzymatic activities. The brain and the pituitary are derived from a common ectodermic origin (10, 11). The pituitary secretes several peptide hormones and also plays a central role in regulating both the nervous and endocrine systems. The latter includes endocrine glands such as the thyroid, pancreas, adrenal medulla, and hypothalamus.

This study was conducted to explore the expression and localization of IA-2 mRNA in the adult mouse brain and pituitary. In addition, Northern blot analysis, RT-PCR analysis and the nonradioactive *in situ* hybridization technique were conducted to study the

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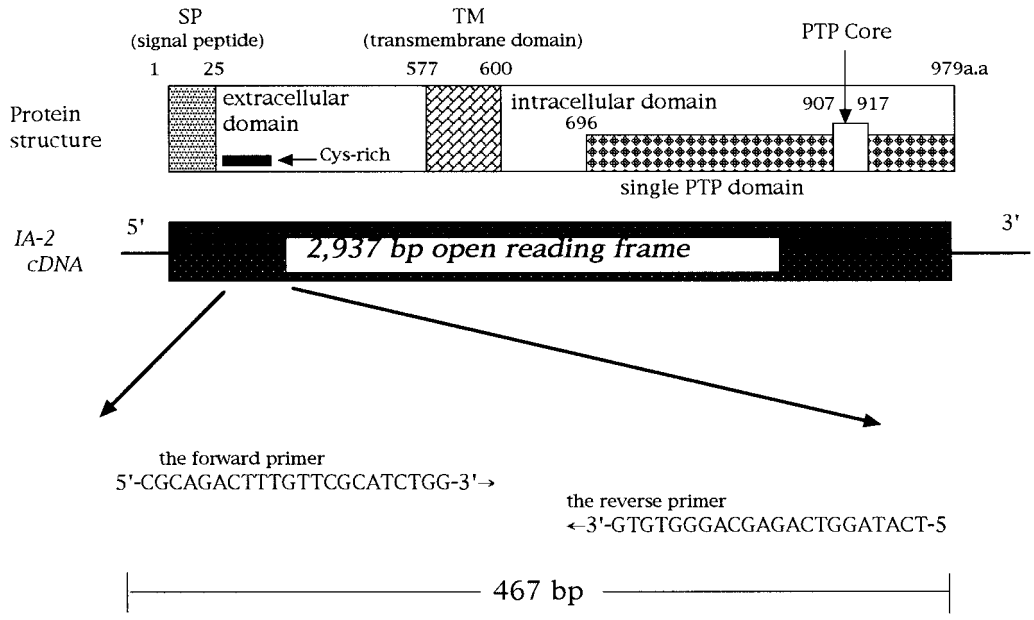

FIG. 1. (Top) Structural features of transmembrane receptor-type protein tyrosine phosphatase. (Bottom) Full-length IA-2 cDNA. Primers were designed to amplify part of the IA-2 open-reading frame, which is a part of the extracellular domain with a unique cystein-rich segment, based on known sequences of mouse IA-2 cDNA. The expected length of the products by RT-PCR is 467 bp.

IA-2 mRNA expression patterns in the brain and pituitary in 1-, 4-, and 8-week-old mice.

MATERIALS AND METHODS

Animals. One-, four -, and eight-week-old male BALB/cA Jcl mice (Clea Japan Inc., Tokyo, Japan) were maintained under routine laboratory conditions (23 ± 2°C; relative humidity, 55 ± 5%) with free access to animal chow and drinking water until use.

RNA isolation. Total RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform (AGPC) extraction method (12). The pancreas was removed and immediately frozen in solution D containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosil, and 0.1 M 2-mercaptoethanol at −80°C. Frozen tissues were gently homogenized with a glass-teflon homogenizer in 20 ml of solution D and subsequently transferred to a 50-ml polypropylene tube. Then, 2 ml of 2 M sodium acetate (pH 4.0), 20 ml of phenol equilibrated with water, and 4 ml of chloroform-isoamyl alcohol (24:1) were added to the homogenate sequentially with inversion-mixing after the addition of each reagent. The final suspension was shaken vigorously for 10 s and cooled on ice for 15 min. After the sample was centrifuged (10,000*g* for 20 min at 4°C), the aqueous phase was transferred to a fresh tube, precipitated with an equal volume of isopropanol and allowed to stand at −20°C for at least 1 h to precipitate RNA before further centrifugation (10,000*g* for 20 min at 4°C). The RNA pellet was redissolved in 5 ml of solution D and reprecipitated with isopropanol (5 ml). The resulting pellet was washed twice with 75% ethanol and dried. The RNA pellet was dissolved in RNase-free water before overnight treatment with diethyl pyrocarbonate (DEPC) and subsequent sterilization with an autoclave.

The tissues, except for pancreas, were snap-frozen in liquid nitrogen and stored at −80°C until use. Total RNA from frozen surgical specimens was extracted using the Trizol reagent (Gibco BRL Life Technologies Inc., Gaithersburg, MD), a commercially available phenol/guanidinium isothiocyanate mixture, following the manufacturer's instructions (improved with the AGPC isolation method).

The RNA concentration was estimated by electrophotometric analysis at 260 nm. The RNA integrity was assessed by running the RNA on an agarose gel followed by visualization of the 18s and 28s rRNA bands with ethidium bromide.

Primer design. Two primers were designed to amplify part of the coding region of the unique extracellular domain containing a cystein-rich segment of IA-2 (Fig. 1). The amplified DNA fragment (467 bp) corresponded to nucleotides 139 to 605 of the IA-2 cDNA sequence based on the known sequence of mouse full-length IA-2 cDNA (GenBank Accession No. U11812-2) (7). Forward 5′-CGCAGACTTTGTTCGCATCTGG-3′ and Reverse 5′-TCATAGGTCAGAGCAGGGTGTG-3′.

Reverse transcriptase-polymerase chain reaction (RT-PCR). To convert RNA to cDNA, RNA (1 μg) from mouse tissues was prepared as described above and was reverse transcribed in a total volume of 20 μl comprising 10 mM Tris–HCl (pH 8.3), 5 mM $MgCl_2$, 50 mM KCl, 20 units of cloned moloney murine leukemia virus reverse transcriptase (United States Biochemical Corp., Cleveland, OH), and 2.5 M oligo $d(T)_{16}$ primer. The sample was layered with 50 μl of mineral oil. After incubation at 37°C for 60 min, the reaction sample (4 μl) for PCR (25 μl) employed a reaction cocktail incubated with 50 pmol of each primer, 2.5 u of Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT), 10 mM Tris–HCl (pH 8.6), 50 mM KCl, 1.5 mM MgCl2, and 0.2 mM dNTPs. IA-2 cDNA was amplified for 35 cycles using the Program Temp-Control System PC-700 (Astec Inc., Fukuoka, Japan). The PCR reactions consisted of 60 s at 94°C (denaturing), 90 s at 68°C (annealing), and 90 s at 72°C (extending).

Electrophoretic analysis of PCR products. A portion (20 μl) of the PCR reaction mixture containing the amplified DNA fragments was fractionated by electrophoresis on a TBE 2% agarose gel containing 0.5 μg/ml ethidium bromide and DNA identification was performed under ultra-violet (UV) light.

Sequencing of PCR fragments. After agarose gel electrophoresis, the PCR products corresponding to the predicted amplified IA-2 cDNA fragments were excised under UV light and isolated using a Prep-A-Gene DNA purification Matrix (Bio-Rad Laboratories, Rich-

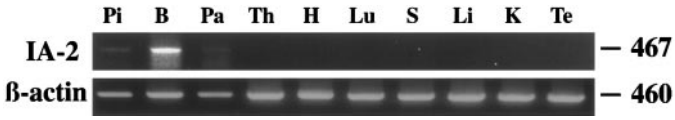

FIG. 2. RT-PCR analysis on the specific expression of the IA-2 mRNA in multiple normal male mouse tissues. Total RNAs (1 μg) were subjected to reverse transcription and PCR amplification (35 cycles). The arrow-labeled IA-2 and fl-actin indicate predicted 467 and 460 bp fragments, respectively. The reactive tissue source of the mRNA in each lane is pituitary (pi), brain (B), pancreas (P), thymus (Th), heart (H), lung (Lu), spleen (S), liver (Li), kidney (K), testis (Te). Molecular weight marker (MW), a *Hin*dIII, *Eco*RI digested DNA.

mond, CA). The purified DNA fragment was directly inserted into a pT7Blue T-Vector (Novagen) using the DNA ligation kit (Takara, Shiga, Japan) for cloning and sequencing. Plasmid DNA samples were then prepared with a Qiagen plasmid mini kit (Funakoshi, Tokyo, Japan). The nucleotide sequence was determined by the dideoxy chain-termination method using a Dye terminator cycle sequencing kit (Perkin Elmer Co., Chiba, Japan) in an ABI PRISMTM310 genetic analyzer (Perkin Elmer Co., Chiba, Japan). Sequence data was processed with DENASISTM-MAC (Hitachi Co., Ltd., Kanagawa, Japan) on a Macintosh personal computer.

Preparation of fluorescein-labeled probes. The RT-PCR product was subcloned into pT7Blue T-Vectors (Novagen). After the determination of nucleotide sequence, PCR was performed by using the vector containing the IA-2 cDNA fragment as a template. PCR was carried out at a reaction volume of 50 μl containing 2.5 u of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs, and 50 pmol of forward and reverse primers overlaid with mineral oil, (Program Temp Control System PC-700; Astec Inc., Fukuoka, Japan). Amplification was set at 94°C for 60 s, 68°C for 90 s, and 72°C for 90 s followed by a 20-min extension at 72°C for 35 cycles. Part of the first PCR product (1 μl) was again amplified using the same set of primers (described above). The PCR amplified DNA fragments were labeled with fluorescein(FI)-dUTP (Amersham, Arlington Heights, IL), according to the manufacturer's instruction.

Poly(A)$^+$RNA preparation. Total RNA from each organ was prepared as described above. Poly (A)+RNA was purified using Oligotex-dT30 (Daiichi Pure Chemicals, Tokyo, Japan) as described previously (13).

Nonradioactive Northern blot analysis. Poly(A)$^+$RNA was electrophoresed overnight on a 1% agarose gel containing 5.4% formaldehyde and 1× Mops before transferring to a Hybond-N+ membrane (Amersham International plc, Buckinghamshire, England) by capillary blotting with 3 M NaCl, 10 mM NaOH. After baking the mem-

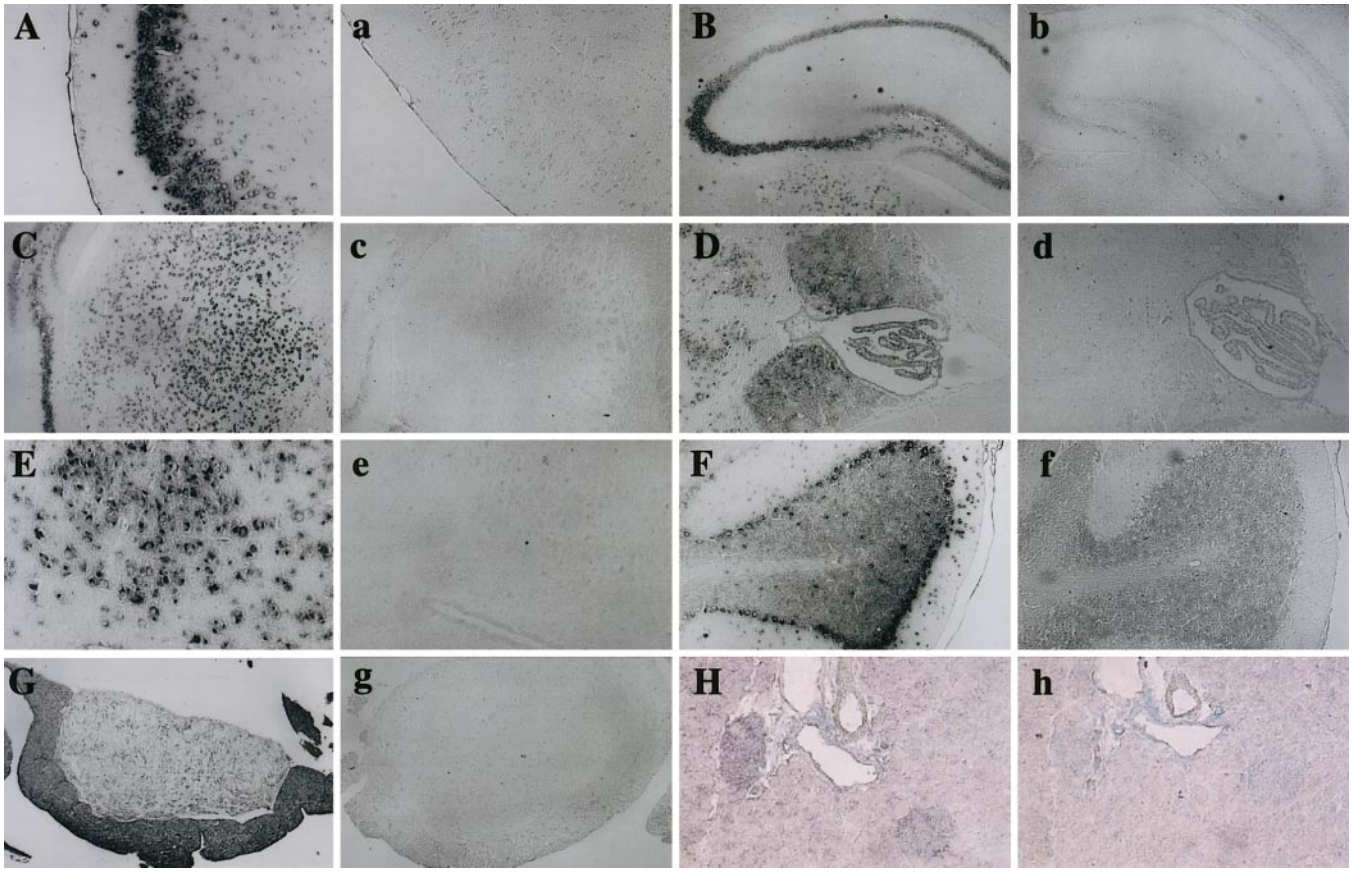

FIG. 3. *In situ* hybridization histochemistry of IA-2 mRNA expression in the normal 8-week-old normal male mouse brain and pituitary. Paraffin sections (4 μm) were pretreated and hybridized with Digoxigenin-11-UTP-labeled antisense (a) RNA probe. After hybridization, the sections were washed and immunodetection was performed to visualize the signal. As a control, sections were hybridized with sense (b) RNA probe under the same conditions and these sections did not show any signals. (A) cerebral cortex, (B) hippocampus, (C) thalamus, (D) choroid plexus and cells of the thalamus, (E) hypothalamus, (F) Purkinje cell layer and granular cell layer of cerebellum, (G) pituitary, and (H) pancreatic islets.

brane for 2 h at 80°C, the filter was prehybridized in 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M 3 Na-citrate, pH 7), 0.7% (w/v) sodium dodecyl sulfate (SDS), 50% deionized formamide, a blocking agent (Amersham International), 50 mM SPB, 0.1% Sarcosyl, and 50 ng/ml yeast tRNA for 2 h at 50°C. Hybridization was performed with FI-dUTP (Amersham International) labeled denatured probe for more than 9 h at 50°C. The membrane was washed twice in 2× SSC/0.1% SDS at room temperature for 5 min and twice in 0.1× SSC/0.1% SDS at 68°C for 15 min. The hybridized RNA bands were detected with alkaline phosphatase conjugated anti-FI antibodies and visualized with the chemiluminescent substrate, CDP-Star (Amersham International) according to the manufacturer's recommendations before exposure to Hyperfilm MP (Amersham International).

Preparation for digoxigenin-labeled probes. Both sense and antisense RNA probes were prepared using a digoxigenin RNA-labeling kit (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany). The RT-PCR products were subcloned into pT7Blue T-Vectors (Novagen) in two directions. These plasmids were linealized with BamHI and then transcribed with T7 RNA polymerase to generate 467-bp antisense probes or sense probes.

Tissue preparation for in situ hybridization. After perfusing with 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4), each organ was dissected and immediately immersed in 4% PFA for 2 h at 4°C. Specimens were dehydrated in graded ethanol, embedded in paraffin, and sectioned at 4 μm by a standard rotary microtome before placement on slides coated with Vectabond (Funakoshi Co. Ltd., Tokyo, Japan). Slides were treated as described previously (14). Briefly, tissue sections were deparaffinized, rehydrated by graded ethanol, and treated with 0.3% Triton X-100 in phosphate-buffered saline for 10 min. Subsequently, sections were treated with 0.2 N HCl in distilled water for 20 min and 2 μg/ml proteinase K in phosphate-buffered saline for 30 min. Further treatments with 0.1 M triethanolamine-HCl (pH 8.0) (TEA) for 1 min and 0.25% acetic anhydride in TEA for 10 min were followed up before rinsing in distilled water for 5 min. The treated sections were then dehydrated and air-dried.

In situ hybridization. The hybridization mixture consisted of 0.5 μl of the digoxigenin-labeled probe suspended in 125 μl hybridization buffer containing 10% dextran sulfate, 5% deionized formamide, 1× Denhardt's solution, 0.33 M NaCl, 2.7 mM EDTA, 22 mM Tris buffer (pH 7.6), and 1 mg/ml of yeast tRNA (Gibco-BRL Life Technologies Inc., Gaithersburg, MD). The probe was denatured by boiling at 85°C for 10 min and rapidly cooled on ice. A volume of 50 μl was dripped on each section before the samples were covered with parafilm and hybridized at 50°C for 16 h in a humid chamber.

After hybridization, the sections were soaked in 5× SSC prior to washing in a solution containing 50% deionized formamide and 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M 3Na-citrate, pH 7.0) at 50°C. RNase A treatment (1 μg) was performed at 37°C for 30 min. These sections were then treated twice with 2× SSC and 0.2× SSC for 30 min at 50°C. The immunological reaction of the digoxigenin-labeled RNA probe-mRNA complex was performed with a solution containing the alkaline phosphatase-labeled anti-digoxigenin antibody Fab fragment (1:500; Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) at 4°C for 16 h after blocking with Tris buffer II (1% BSA, 0.3% Tween-20 in Tris buffer I) for 1 h. The antibody conjugate was diluted with 0.1 M Tris buffer I containing 100 mM Tris–HCl (pH 7.5) and 150 mM NaCl. After thorough rinsing in 0.1 M Tris buffer I (3 times for 15 min) and Tris buffer III (100 mM Tris–HCl pH 7.5, 100 mM NaCl, 50 mM $MgCl_2$) for 5 min, an alkaline phosphate complex was revealed when freshly prepared solution of 45 μl Nitro blue tetrazolium (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) and 35 μl 5-bromo-4-chloro-3-indolyl phosphate (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany) in 10 ml Tris buffer III were added. After the reaction, the samples were rinsed with distilled water and counterstained with methylgreen.

Tissue sections were hybridized with a digoxigenin-labeled antisense RNA probe. In addition, hybridization was also performed with a sense-stranded probe derived from the same region to confirm specificity of the observed signals.

RESULTS

To study the tissue-specific expression of IA-2 mRNA, RT-PCR was used to detect IA-2 mRNA in 8-week-old normal male mouse tissues, including pituitary, brain, pancreas, thymus, heart, lung, spleen, liver, kidney, and testis (Fig. 2). The amplified RT-PCR products were electrophoresed on a 2% agarose gel. Total RNAs from mouse tissues resulted in the amplification of products with the size (467 bp) previously predicted by RT-PCR. RT-PCR analysis showed that IA-2 mRNA was detected in the pituitary, brain, and pancreas. The RT-PCR products were sequenced and confirmed to be products from IA-2 m RNA. The highest expression of IA-2 mRNA was detected in the brain, followed by the pituitary and pancreas.

As described above, IA-2 mRNA was expressed in the brain and pituitary by RT-PCR. To more precisely localize the expression in the 8-week-old male mouse brain and pituitary, *in situ* hybridization was performed. IA-2 mRNA was expressed markedly in the neurons located within the cerebral cortex (Fig. 3A). In the hippocampus, IA-2 mRNA was expressed in all pyramidal cells in fields CA1, CA2, and CA3 and dentate gyrus hillar cells (Fig. 3B). Pyramidal cells in the region of CA2 showed strong signals. IA-2 mRNA expressions in the thalamus (Fig. 3C), choroid plexus (Fig. 3D), and hypothalamus (Fig. 3E) were observed. In the cerebellum (Fig. 3F), IA-2 mRNA was strongly expressed in the Purkinje cells and weakly expressed in the granule cell layer of the cerebellar cortex. In the brain stem and spinal cord (data not shown), IA-2 mRNA signals were strongly expressed in larger nerve cells. In the pituitary (Fig. 3G), IA-2 mRNA was expressed markedly in the anterior pituitary and weakly in the posterior pituitary. In the pancreas, localization of IA-2 mRNA was demonstrated in the islets of Langerhans of 8-week-old mice (Fig. 3H). IA-2 mRNA was detectable using hybridization with the antisense probe, although hybridization was negative with the sense probe. Using immunohistochemistry, these IA-2 mRNA-positive cells, coinciding with alpha, beta, and delta cells in the islets, were stained with anti-insulin, glucagon, and somatostatin antibodies (data not shown).

To study the pattern of expression of IA-2 mRNA in the brain, Northern blot analysis was performed by using poly $(A)^+$RNAs prepared from the whole brain, cerebrum, cerebellum, brain stem, and spinal cord of 1-, 4-, and 8-week-old normal male mice. IA-2 mRNA was detected in all brain samples from mice at 1, 4, and 8 weeks of age. The size of the IA-2 mRNA observed in

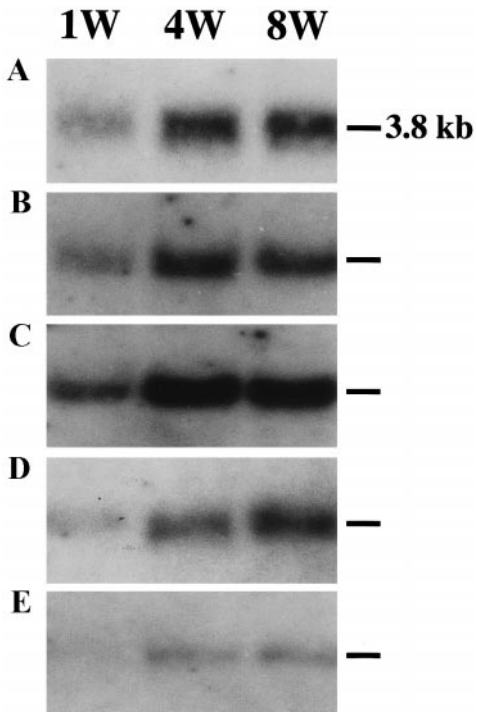

FIG. 4. Northern blot analysis of IA-2 mRNA expression in brain and spinal cord. Poly(A)$^+$ mRNA (9.5 μg) was extracted from whole brain of normal 1-, 4-, and 8-week-old male mice (A). Northern blot analysis of IA-2 mRNA expression in various anatomical regions of the brain. Thirteen micrograms of Poly(A)$^+$ mRNA was extracted from normal 1-, 4-, and 8-week-old mouse cerebra (B). Ten micrograms of Poly(A)$^+$ mRNA was extracted from normal 1-, 4-, and 8-week-old mouse cerebella (C). Three micrograms of Poly(A)$^+$ mRNA was extracted from normal 1-, 4-, and 8-week-old mouse brain stems (D). Three micrograms of Poly(A)$^+$ mRNA was extracted from normal 1-, 4-, and 8-wk-old mouse spinal cords (E). Samples of Poly(A)$^+$ mRNA from the indicated tissues were electrophoresed on a 1% agarose/formaldehyde gel, transferred to a nylon membrane and hybridized with FITC-labeled IA-2 cDNA probe to code for part of the extracellular domain. Message size of IA-2, 3.8 kb (arrow) was estimated by ribosomal RNAs. The membrane was exposed for 1 h (A and D), and 30 min (B, C, and E).

all tissues was 3.8 kb. In the whole brain (Fig. 4-A) the level of IA-2 mRNA expression was higher at 4 weeks and lower at 1 week of age. In the cerebrum (Fig. 4B), and cerebellum (Fig. 4C), the levels of IA-2 mRNA expression were also higher at 4 weeks and lower at 1 week of age. In the brain stem (Fig. 4D), the level of IA-2 mRNA expression was higher at 8 weeks and lower at 1 week of age. In the spinal cord (Fig. 4E), the level of IA-2 mRNA expression was extremely low at 1 week of age.

To study the pattern of expression of IA-2 mRNA in pituitary, RT-PCR was performed to detect IA-2 mRNA in 1-, 4-, and 8-week-old normal male mice (Fig. 5). The amplified RT-PCR products were electrophoresed on a 2% agarose gel. Total RNAs from all samples resulted in the amplification of products with a size (467 bp) previously predicted by RT-PCR. The electrophoresis results of RT-PCR products were quantitatively analyzed using a microcomputer-based NIH-image analysis system, and the relative expression levels of IA-2 mRNA were determined. The relative expression levels were normalized with reference to those of fl-actin mRNAs. Faint levels of IA-2 mRNA expression were detected at 1 week of age. Compared to the basal levels at 1 week of age, expression increased 4.2-fold at 4 weeks and 1.88-fold at 8 weeks of age.

IA-2 mRNA expressions in pancreas of 1-, 4-, and 8 week-old mice were studied by RT-PCR analysis. The data in the gel electrophoresis revealed that the expression of IA-2 mRNA from 1-week-old mice was lower than that of ages 4 and 8 weeks (Fig. 5). Analysis of the level of IA-2 mRNA expression by the microcomputer-based NIH-image analysis system showed the intensity ratios were 1.00, 1.67, and 1.84 in 1-, 4-, and 8-week-old mice, respectively. Beta-actin mRNA was used as a basal reference control. These results were calculated based on the average of three mice from each group.

DISCUSSION

The expression of IA-2 mRNA was investigated by RT-PCR using RNA from tissues of 8-week-old normal male mice. The amplification of predicted DNA size (467 bp) was observed in the mouse brain, pancreas, and pituitary. IA-2 mRNA was not detected in other normal tissues including thymus, heart, lung, spleen, liver, kidney, and testis. Using cloned IA-2 cDNA as hybridization probes in Northern blot analyses, IA-2 mRNA was detected in normal human brain, pancreas, and pituitary (7) and in normal murine brain, pancreas, and some gastrointestinal tissues (15). IA-2 mRNA was not detected in other tissues such as the liver, lung, spleen, thymus, and kidney by Northern blot analyses as described above. Ongoing studies in our laboratory attempt to develop anti-mouse IA-2 antibodies using IA-2 gene-deficient mice. However, development of highly specific anti-mouse IA-2 antibodies is our current high-priority issue.

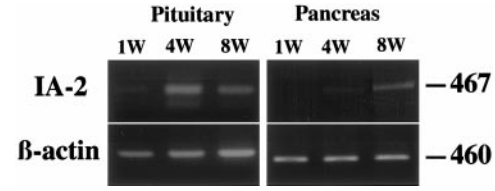

FIG. 5. Patterns of IA-2 mRNA expression in normal male mice pituitary and pancreas. RT-PCR analysis was performed of the total RNA (1 μg) from the normal 1-, 4-, and 8-wk-old male mouse pituitary, respectively (35 cycles). The arrow labeled IA-2 and fl-actin indicate predicted 467 and 460 bp fragments, respectively. The tissue source of the mRNA in each lane is 8-week-old mouse (8w), 4-week-old mouse (4w), and 1-week-old mouse (1w). Molecular weight marker (MW), a *Hin*dIII, *Eco*RI digested DNA.

In the present study, Northern blot analysis was performed to detect the distribution of IA-2 mRNA in certain anatomical regions (cerebrum, cerebellum, brain stem, and spinal cord) dissected from 8-week-old normal male mouse brain. Northern blot analysis showed that IA-2 mRNA was expressed in all tissues examined and the message sizes of IA-2 convergently approximated to 3.8-kb mRNA. Our results suggest that IA-2 mRNA was expressed strongly in the cerebrum and weakly in the cerebellum. Relative levels of IA-2 mRNA expression might differ in different neuronal tissues. *In situ* hybridization revealed that IA-2 mRNA was expressed in neurons of the cerebral cortex, hippocampus, thalamus, hypothalamus, choroid plexus, Purkinje cells, and granule cell layer of the cerebellar cortex of 8-week-old normal male mice. IA-2 mRNA was expressed in the anterior and posterior regions of 8-week-old mouse pituitary. Our results were consistent with the concept that IA-2 was expressed predominantly in cells of neuroendocrine origin (7, 16, 17). In addition, IA-2 mRNA was localized in alpha, beta, and delta cells of mouse pancreatic islets.

The major antigenic determinant of IA-2 is located at the COOH-terminal of the intracellular domain of IA-2, although IA-2 is a transmembrane PTP (18–20). IA-2 may be recognized as a consequence of beta-cell destruction. Furthermore, distribution of IA-2 in rat tissues by immunocytochemistry was restricted to neuroendocrine cells (16). By immunoelectron microscopy on ultrathin cryosections of rat posterior pituitary, IA-2 was found to be localized in secretory granules (21). Taken together, these reports demonstrate that IA-2 mRNA might be expressed in hormone-secreting endocrine cells and neurons containing neurosecretory granules within the brain and pituitary.

The level of IA-2 mRNA expression was higher in 4-week-old normal mice and lower in 1-week-old mice in the whole brain, cerebrum, and cerebellum. The pattern of IA-2 mRNA expression in the pituitary was investigated by RT-PCR. The amplification of predicted DNA size (467 bp) was observed, indicating that IA-2 mRNA was present in 1-, 4-, and 8-week-old normal male mouse pituitary. The levels of IA-2 mRNA expression were higher at 4 weeks and lower at 1 week of age. The levels of IA-2 mRNA expression in pancreatic islets in 1-week-old mice were lower than those in 4- and 8-week-old mice. Ongoing experiments show that IA-2 mRNA expression is lower in pancreatic islets of 1-week-old NOD mice. In 4-week-old NOD female mice, the expression level was higher than that in BALB/c mice. Autoimmune insulitis was observed from 8 weeks of age in NOD mice (data not shown). IDDM in mice was observed at 8 weeks of age after IA-2 mRNA expression was enhanced. At 14 weeks of age, after IDDM was observed, IA-2 mRNA expression levels were variable.

In a previous study, expression of the human pancreatic autoantigen ICA69 increased with age, levels in adults being fivefold higher than those registered at 13 weeks of gestation (22). The mechanism and meaning of the developmental increase in autoantigen is not well understood. In our experiment, data from Northern blot and RT-PCR analyses suggest that the amount of IA-2 mRNA expression increased in accordance with development of the brain, pituitary, and pancreas. Further studies on the posttreatment expression of IA-2 mRNA in brain and pituitary in association with the trigger mechanism on production, storage, and secretion of hormones and biogenic amines are warranted to understand the function of IA-2 molecules.

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