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Molecular Cloning of Rat Leptin Receptor Isoform Complementary DNAs—Identification of a Missense Mutation in Zucker Fatty (*fa*/*fa*) Rats

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We cloned the full-length rat leptin receptor (Ob-R) isoform complementary DNAs (cDNAs) and examined the gene expression in rats. We also identified a mutation in Ob-R in Zucker fatty (*fa*/*fa*) rats. Three alternatively spliced isoforms (Ob-Ra, Ob-Rb, and Ob-Re) have been identified, which are closely related to the gp130 signal-transduction component of class I cytokine receptors. Rat Ob-Ra and Ob-Rb were single transmembrane proteins, which differ in the C-terminal amino acid sequences. On the other hand, Ob-Re had no transmembrane domain and was a soluble form of the receptor. Reverse transcription-polymerase chain reaction analysis revealed that Ob-R isoform messenger RNAs (mRNAs) are expressed in a wide variety of rat tissues in tissue-specific manners. A missense mutation (an A to C conversion at nucleotide position 806) was found in the extracellular domain of all the isoforms in Zucker fatty (*fa*/*fa*) rats, which resulted in an amino acid change from Gln to Pro at + 269 (the Gln269Pro mutation). These Ob-R isoform mRNAs were present in the brain from Zucker fatty (*fa*/*fa*) rats at comparable amounts to those in their lean littermates. The present study provides new insight into the molecular mechanisms for Ob-R. © 1996 Academic Press, Inc.

The obesity and diabetes syndromes caused by the two single autosomal recessive mutations, *obese* (*ob*) and *diabetes* (*db*) are identical on the same strain background (1). Parabiosis experiments have suggested that *ob*/*ob* mice are unable to produce a circulating satiety factor, while *db*/*db* mice are resistant to it (1). Zhang et al. have recently isolated the *ob* gene (2), which encodes a 166-/167-amino acid polypeptide (leptin) that is expressed specifically in the adipose tissue (2–5). They also found a nonsense mutation in the *ob* gene from C57BL/6J *ob*/*ob* mice (2). It has been shown that synthesis and secretion of leptin are greatly augmented in several models of rodent obesity and human obesity (2, 3, 5–11). Recent studies have demonstrated that recombinant leptin reduces food intake and body weight gain in mice and corrects the obesity-related phenotypes in *ob*/*ob* mice, but not in *db*/*db* mice (12–17). These findings, taken together, indicate that leptin is a fat cell-derived circulating satiety factor, which *ob*/*ob* mice are deficient in, and *db*/*db* mice cannot respond to.

Tartaglia et al. have isolated mouse and human leptin receptor (Ob-R) complementary DNAs (cDNAs) (18). The receptor is a single transmembrane protein closely related to the gp130 signal-transduction component of class I cytokine receptors (18). The receptor, the *db* gene product, has several alternatively spliced isoforms, which are expressed in a wide variety of tissues (18–22). The longest isoform (Ob-Rb) is expressed at a high level in the hypothalamus, and is missing in *db*/*db* mice due to a mutation leading to an abnormal transcript of Ob-Rb (19, 20). These findings suggest that loss of satiety effect of leptin in *db*/*db* mice is due to a

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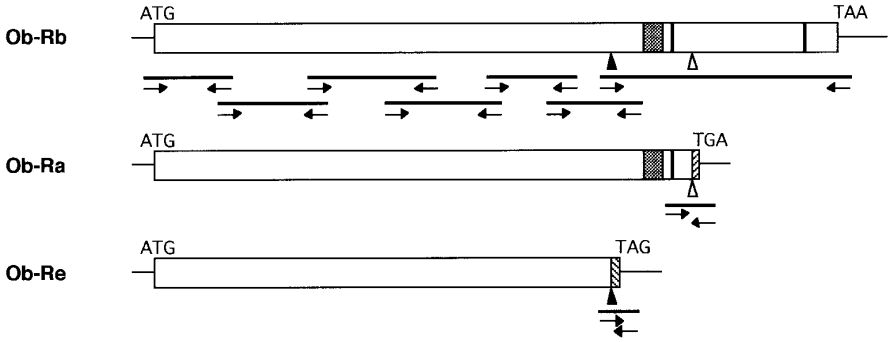

FIG. 1. Schematic representation of rat Ob-Rb, Ob-Ra, and Ob-Re isoform cDNAs. The coding regions of Ob-R isoforms are depicted by boxes. Sites of PCR primers (arrows) and RT-PCR products (thick bars) are indicated below each isoform. Sites of alternative mRNA splicing for Ob-Re, and for Ob-Rb and Ob-Ra are indicated by closed and open arrowheads, respectively. Transmembrane domains of Ob-Rb and Ob-Ra are indicated by dotted boxes. Ob-Ra and Ob-Re isoform-specific sequences are depicted by striped boxes. The Box 1 and Box 2 consensus sequences are indicated by closed boxes.

defect in interactions between leptin and its receptor in the hypothalamus. The rat gene *fatty* (*fa*) has been shown to be a homologue of the mouse *db* gene (23), and it has been suggested that *fa*/*fa* rats also develop obesity and diabetes due to a mutation in the Ob-R gene locus (21). To further understand the molecular mechanisms for action of leptin, in the present study, we have cloned the full-length rat Ob-R isoform cDNAs and examined expression of each isoform mRNA in rats. We have also identified a mutation in the coding region of Ob-R from genetically obese Zucker fatty (*fa*/*fa*) rats.

MATERIALS AND METHODS

Animals. Ten-week-old male Sprague-Dawley rats and 23-week-old Zucker fatty (*fa*/*fa*) rats and their lean littermates were purchased from Shimizu Experimental Supplies, Kyoto, Japan. The animals were housed in a temperature-, humidity-, and light-controled room (12-h light/12-h dark cycle) and allowed free access to water and standard rat chow (CE-2, 352 kcal/100 g, Japan CLEA, Tokyo, Japan).

Tissue preparation and total RNA extraction. After overnight fast, rats were sacrificed by decapitation. Tissues were removed immediately from rats, frozen in liquid nitrogen, and stored at −70 °C until use. Total RNA was extracted as previously described (3, 4).

Molecular cloning. Based on the nucleotide sequences of mouse Ob-Ra, Ob-Rb, and Ob-Re cDNAs (18–20), several sets of polymerase chain reaction (PCR) primers were generated by a Model 381A DNA synthesizer (Applied Biosystems Inc., Foster City, CA) (Fig. 1). Ten μg of total RNA from the rat brain was reverse-transcribed by random hexamer priming using Superscript Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Gaithersburg, MA). The single stranded cDNAs were subjected to PCR as described (24). Nine reverse transcription-PCR (RT-PCR) products (Fig. 1) were subcloned into the pCR II vector (Invitrogen Corp., San Diego, CA) for sequencing. To exclude the nucleotide misincorporation during PCR amplification, RT-PCR was also performed using rat Ob-R sequence-specific primers, which was subjected to the direct sequencing.

DNA sequencing. Sequence determination was carried out by the dideoxy chain termination method (3, 4, 24) using Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, OH). Direct sequencing of the RT-PCR products was performed using PRISM sequenase fluorescent dye-labeled dideoxy-nucleotide kit (Applied Biosystems Inc.). All DNA sequences were confirmed by reading both DNA strands.

Gene expression analysis. To elucidate Ob-Ra and Ob-Rb isoform mRNA expression in rats, RT-PCR was also carried out using a common sense primer and isoform-specific antisense primers (sense: 5′-ATGAAGTGGCTTAGA-ATCCCTTCG-3′; antisense: 5′-TACTTCAAAGAGTGTCCGCTC-3′ for Ob-Ra, and 5′-ATATCACTGATTCTG-CATGCT-3′ for Ob-Rb). For Ob-Re isoform, RT-PCR was done using isoform-specific sense and antisense primers (sense: 5′-TTCCTGTGGGCAGAATCAGCACACACTGTT-3′, and antisense: 5′-AAGCACAGTACACATACC-3′). The expected sizes of RT-PCR products for Ob-Ra, Ob-Rb, and Ob-Re were 347 basepair (bp), 375 basepair (bp),

and 305 basepair (bp), respectively. RT-PCR was also performed using primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Clontech Laboratories Inc., Mountain View, CA) to verify the integrity of RNA used.

Identification of a mutation in Zucker fatty (fa/fa) rats. To identify the possible mutation in the entire coding region of Ob-R isoforms in Zucker fatty (*fa*/*fa*) rats, RT-PCR with several sets of primers for the entire coding region of Ob-R cDNAs was performed using RNA from the brain form 23-week-old Zucker fatty (*fa*/*fa*) rats and their lean littermates. Direct sequencing of the RT-PCR products were carried out as described above.

RESULTS

Molecular cloning of rat Ob-R isoform cDNAs. To determine the nucleotide sequences of the entire coding regions of rat Ob-R isoform cDNAs, RT-PCR was performed using total RNA from the rat brain as template (Fig. 1). Rat Ob-R was a single transmembrane protein, and was closely related to gp 130 of class I cytokine receptor (25). The N-terminal hydrophobic 22-amino-acid peptide represented the signal sequence (Fig. 2). In the extracellular domain of rat Ob-R, there were two W-S-X-W-S motifs (where X is a nonconserved amino acid), which are involved in the ligand binding (25). Rat Ob-Ra and Ob-Rb were 894 and 1162 amino acid long, respectively, and differed in the C-terminal amino acid sequences. Rat Ob-Rb had the longer intracellular domain with both the Box 1 and Box 2 consensus sequences, which are involved in interactions with Janus protein-tyrosine kinases (JAKs) and signal transducers and activators of transcription (STATs), respectively (25). Rat Ob-Ra was identical to rat Ob-Rb upstream of the Lys residue at + 889, and differed downstream of it at the C-terminal region (Figs. 1 & 2). Accordingly, rat Ob-Ra contained only the Box 1 sequence in its intracellular domain. By contrast, rat Ob-Re, a 805 amino acid protein, had no transmembrane domain, suggesting that it is a soluble form of the receptor (Fig. 2). Analysis of the deduced amino acid sequences revealed that rat Ob-Rb is 91 % and 75 % identical to mouse and human Ob-Rb, respectively. Rat Ob-Ra was 93 % and 77 % identical to mouse and human Ob-Ra, respectively (Fig. 3). Rat Ob-Re had 92 % identity with the mouse counterpart (Fig. 3).

Expression of Ob-R isoform mRNAs in Sprague-Dawley rats. RT-PCR analysis using isoform-specific primers revealed that Ob-Ra, Ob-Rb, and Ob-Re mRNAs are expressed in a wide variety of rat tissues (Fig. 4A). Ob-Rb mRNA was expressed at the highest level in the brain, at moderate levels in the lung, kidney, small intestine, spleen, testis and fat tissue, and at a low level in the stomach. No significant amount of Ob-Rb mRNA was detected in the heart and liver. Ob-Ra mRNA was present at high levels in the brain, kidney, small intestine, spleen, testis, and fat tissue and at low levels in the lung, stomach, and liver. A little amount of Ob-Ra mRNA was detectable in the heart. Ob-Re mRNA was also expressed at a high level in the brain followed by the lung, kidney, small intestine, spleen, testis, and fat tissue. No appreciable amount of Ob-Re mRNA was detected in the heart, stomach, and liver. To further examine expression of these Ob-R isoform mRNAs in the brain, RT-PCR was performed using total RNA from the hypothalamus, cerebral cortex, and cerebellum (Fig. 4B). Ob-Rb and Ob-Ra mRNAs were detected at high levels in all the brain regions examined. Ob-Re mRNA was also detected in these brain regions, but at relatively low levels.

Identification of a mutation in Ob-R isoform from Zucker fatty (fa/fa) rats. To identify the possible mutation of Ob-R cDNA in Zucker fatty (*fa*/*fa*) rats, the nucleotide sequences of the entire coding regions of Ob-R isoform cDNAs from Zucker fatty (*fa*/*fa*) rats were determined and compared with those from their lean littermates. A single missense mutation (an A to C conversion at nucleotide 806) was found in the extracellular domain common to all the Ob-R isoforms in Zucker fatty (*fa*/*fa*) rats as compared with their lean littermates (Fig. 5A). The mutation resulted in an amino acid change from Gln to Pro at + 269 (the Gln269Pro mutation).

Expression of Ob-R isoform mRNAs in Zucker fatty (fa/fa) rats. To examine whether Ob-R mRNAs are expressed in these animals, we also examined Ob-R gene expression in the brain from Zucker fatty (*fa*/*fa*) rats. RT-PCR analysis revealed that three Ob-R isoform mRNAs

Ob-Rb

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GTGTCTATCTCTGAAGTAAG -1
ATGACGTGTCAGAAATTCTATGTGGTTTTGTTACACTGGGAATTTCTGTATGTGATAACTGCACTTAACCTGGCCTATCCAACCTCTCCCTGGAGATTTAAGCTG 105
 M  T  C  Q  K  F  Y  V  V  L  L  H  W  E  F  L  Y  V  I  T  A  L  N  L  A  Y  P  T  S  P  W  R  F  K  L 35
TTTTGTGCGCCACCGAGTACAACTGATGACTCCTTTCTCTCTCCTGCTGGAGTCCCAAACAATACTTCGTCTTTGAAGGGGGCTTCTGAAGCACTTGTTGAAGCT 210
 F  C  A  P  P  S  T  T  D  D  S  F  L  S  P  A  G  V  P  N  N  T  S  S  L  K  G  A  S  E  A  L  V  E  A 70
AAATTTAATTCAACTGGTATCTACGTTTCTGAGTTATCCAAAACCATTTTCCACTGTTGCTTTGGGAATGAGCAAGGTCAAAACTGCTCCGCACTCACAGGCAAC 315
 K  F  N  S  T  G  I  Y  V  S  E  L  S  K  T  I  F  H  C  C  F  G  N  E  Q  G  Q  N  C  S  A  L  T  G  N 105
ACTGAAGGGAAGACGCTGGCTTCAGTGGTGAAGCCTTTAGTTTTCCGCCAACTAGGTGTAAACTGGGACATAGAGTGCTGGATGAAAGGGGACTTGACATTATTC 420
 T  E  G  K  T  L  A  S  V  V  K  P  L  V  F  R  Q  L  G  V  N  W  D  I  E  C  W  M  K  G  D  L  T  L  F 140
ATCTGTCATATGGAACCATTACTTAAGAACCCCTTCAAGAATTATGACTCTAAGGTTCACCTTTTATATGATCTGCCTGAAGTTATAGATGATTTGCCTCTGCCC 525
 I  C  H  M  E  P  L  L  K  N  P  F  K  N  Y  D  S  K  V  H  L  L  Y  D  L  P  E  V  I  D  D  L  P  L  P 175
CCACTGAAAGACAGCTTTCAGACTGTCCAGTGCAACTGCAGTGTTCGGGAATGCGAATGTCATGTACCAGTACCCAGAGCCAAAGTCAACTACGCTCTTCTGATG 630
 P  L  K  D  S  F  Q  T  V  Q  C  N  C  S  V  R  E  C  E  C  H  V  P  V  P  R  A  K  V  N  Y  A  L  L  M 210
TATTTAGAAATCACATCTGCTGGTGTGAGTTTTCAGTCACCTCTAATGTCACTGCAGCCCATGCTTGTTGTGAAGCCCGATCCACCGCTGGGTTTGCGTATGGAA 735
 Y  L  E  I  T  S  A  G  V  S  F  Q  S  P  L  M  S  L  Q  P  M  L  V  V  K  P  D  P  P  L  G  L  R  M  E 245
GTCACAGATGATGGTAATTTAAAGATTTCATGGGACAGCCAAACAAAAGCACCATTTCCACTTCAATATCAGGTGAAATATTTAGAGAATTCTACAATCGTAAGA 840
 V  T  D  D  G  N  L  K  I  S  W  D  S  Q  T  K  A  P  F  P  L  Q  Y  Q  V  K  Y  L  E  N  S  T  I  V  R 280
GAGGCTGCTGAAATCGTCTCGGATACATCTCTGCTGGTAGACAGCGTGCTTCCTGGGTCTTCATACGAGGTCCAGGTGAGGAGCAAGAGACTGGATGGCTCAGGA 945
 E  A  A  E  I  V  S  D  T  S  L  L  V  D  S  V  L  P  G  S  S  Y  E  V  Q  V  R  S  K  R  L  D  G  S  G 315
GTCTGGAGTGACTGGAGTTTACCTCAACTCTTTACCACACAAGATGTCATGTATTTTCCACCCAAAATTCTGACGAGTGTTGGATCCAATGCTTCCTTTTGCTGC 1050
 V  W  S  D  W  S  L  P  Q  L  F  T  T  Q  D  V  M  Y  F  P  P  K  I  L  T  S  V  G  S  N  A  S  F  C  C 350
ATCTACAAAAATGAGAACCAGACTATCTCCTCAAAACAAATAGTTTGGTGGATGAATCTAGCCGAGAAGATCCCCGAGACACAGTACAACACTGTGAGTGACCAC 1155
 I  Y  K  N  E  N  Q  T  I  S  S  K  Q  I  V  W  W  M  N  L  A  E  K  I  P  E  T  Q  Y  N  T  V  S  D  H 385
ATTAGCAAAGTCACTTTCTCCAACCTGAAAGCCACCAGACCTCGAGGGAAGTTTACCTATGATGCAGTGTACTGCTGCAATGAGCAGGCATGCCATCACCGCTAC 1260
 I  S  K  V  T  F  S  N  L  K  A  T  R  P  R  G  K  F  T  Y  D  A  V  Y  C  C  N  E  Q  A  C  H  H  R  Y 420
GCTGAATTATATGTGATCGATGTCAATATCAATATATCATGTGAAACTGACGGGTACTTAACTAAAATGACTTGCAGATGGTCACCCAGCACAATCCAATCACTA 1365
 A  E  L  Y  V  I  D  V  N  I  N  I  S  C  E  T  D  G  Y  L  T  K  M  T  C  R  W  S  P  S  T  I  Q  S  L 455
GTGGGAAGCACTGTGCAGTTGAGGTATCACAGGCGCAGCCTGTACTGTCCCGATAATCCATCTATTCGTCCTACATCAGAGCTCAAAAACTGCGTCTTACAGACA 1470
 V  G  S  T  V  Q  L  R  Y  H  R  R  S  L  Y  C  P  D  N  P  S  I  R  P  T  S  E  L  K  N  C  V  L  Q  T 490
GATGGCTTTTATGAATGTGTTTTCCAGCCAATCTTTCTATTATCTGGCTATACAATGTGGATCAGGATCAACCATTCTTTAGGTTCACTTGACTCTCCACCAACG 1575
 D  G  F  Y  E  C  V  F  Q  P  I  F  L  L  S  G  Y  T  M  W  I  R  I  N  H  S  L  G  S  L  D  S  P  P  T 525
TGTGTCCTTCCTGACTCCGTAGTAAAACCACTACCTCCATCTAATGTAAAAGCAGAGATTACTATAAACACTGGATTATTGAAAGTATCTTGGGAAAAGCCAGTC 1680
 C  V  L  P  D  S  V  V  K  P  L  P  P  S  N  V  K  A  E  I  T  I  N  T  G  L  L  K  V  S  W  E  K  P  V 560
TTTCCAGAGAATAACCTTCAGTTCCAGATTCGATATGGCTTAAATGGAAAAGAAATACAATGGAAGACACACGAGGTATTCGATGCAAAATCAAAATCGGCCAGC 1785
 F  P  E  N  N  L  Q  F  Q  I  R  Y  G  L  N  G  K  E  I  Q  W  K  T  H  E  V  F  D  A  K  S  K  S  A  S 595
CTGCCAGTGTCAGATCTCTGTGCGGTCTATGTGGTACAGGTTCGCTGCCGGCGGTTGGATGGACTAGGGTATTGGAGTAATTGGAGCAGTCCAGCCTACACTCTT 1890
 L  P  V  S  D  L  C  A  V  Y  V  V  Q  V  R  C  R  R  L  D  G  L  G  Y  W  S  N  W  S  S  P  A  Y  T  L 630
GTCATGGATGTAAAAGTTCCTATGAGAGGGCCTGAATTCTGGAGAATAATGGATGGGGATATTACTAAAAAGGAGAGAAATGTCACCTTGCTTTGGAAGCCACTG 1995
 V  M  D  V  K  V  P  M  R  G  P  E  F  W  R  I  M  D  G  D  I  T  K  K  E  R  N  V  T  L  L  W  K  P  L 665
ATGAAAAATGACTCACTGTGTAGTGTGAGGAGGTATGTGGTGAAGCATCGTACTGCCCACAATGGGACATGGTCACAAGATGTGGGAAATCAGACCAATCTCACT 2100
 M  K  N  D  S  L  C  S  V  R  R  Y  V  V  K  H  R  T  A  H  N  G  T  W  S  Q  D  V  G  N  Q  T  N  L  T 700
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 F  L  W  A  E  S  A  H  T  V  T  V  L  A  I  N  S  I  G  A  S  L  V  N  F  N  L  T  F  S  W  P  M  S  K 735
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 V  N  A  V  Q  S  L  S  A  Y  P  L  S  S  S  C  V  I  L  S  W  T  L  S  P  N  D  Y  S  L  L  Y  L  V  I 770
GAATGGAAGAACCTTAATGATGATGATGGAATGAAGTGGCTTAGAATCCCTTCGAATGTTAACAAGTATTATATCCATGATAATTTTATTCCTATCGAGAAATAT 2415
 E  W  K  N  L  N  D  D  D  G  M  K  W  L  R  I  P  S  N  V  N  K  Y  Y  I  H▲ D  N  F  I  P  I  E  K  Y 805
CAGTTTAGTCTTTACCCAGTATTTATGGAAGGAGTTGGAAAACCAAAGATAATTAATGGTTTCACCAAAGATGATATCGCCAAACAGCAAAATGATGCAGGGCTG 2520
 Q  F  S  L  Y  P  V  F  M  E  G  V  G  K  P  K  I  I  N  G  F  T  K  D  D  I  A  K  Q  Q  N  D  A  G  L 840
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 Y  V  I  V  P  I  I  I  S  S  C  V  L  L  L  G  T  L  L  I  S  H  Q  R  M  K  K  L  F  W  D  D  V  P  N 875
CCCAAGAATTGTTCCTGGGCACAAGGACTTAATTTCCAAAAGCCTGAAACATTTGAGCATCTTTTTACCAAGCATGCAGAATCAGTGATATTTGGTCCTCTTCTT 2730
 P  K  N  C  S  W  A  Q  G  L  N  F  Q  KΔ P  E  T  F  E  H  L  F  T  K  H  A  E  S  V  I  F  G  P  L  L 910
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 L  E  P  E  P  V  S  E  E  I  S  V  D  T  A  W  K  N  K  D  E  M  V  P  A  A  M  V  S  L  L  L  T  T  P 945
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 D  S  T  R  G  S  I  C  I  S  D  Q  C  N  S  A  N  F  S  G  A  Q  S  T  Q  G  T  C  E  D  E  C  Q  S  Q 980
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CATTCCCCACTGAGACAGTCTTTTTCTAGCAACTCCTGGGAGATAGAGGCCCAGGCATTTTTCCTTTTATCAGATCATCCACCCAATGTGATTTCACCACAACTT 3150
 H  S  P  L  R  Q  S  F  S  S  N  S  W  E  I  E  A  Q  A  F  F  L  L  S  D  H  P  P  N  V  I  S  P  Q  L 1050
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 S  F  S  G  L  D  E  L  L  E  L  E  G  N  F  P  E  E  N  H  G  E  K  S  V  Y  Y  L  G  V  S  S  G  N  K 1085
AGAGAGAATGATATGCTTTTGACTGATGAGGCAGGGGTATTGTGCCCATTCCCAGCTCACTGTCTGTTCAGTGACATCAGAATCCTCCAGGAGAGTTGTTCACAC 3360
 R  E  N  D  M  L  L  T  D  E  A  G  V  L  C  P  F  P  A  H  C  L  F  S  D  I  R  I  L  Q  E  S  C  S  H 1120
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 F  V  E  N  N  L  N  L  G  T  S  G  K  N  F  V  P  Y  M  P  Q  F  Q  S  C  S  T  H  S  H  K  I  I  E  N 1155
AAGATGTGTGACTTAACTGTGTAATCTTGCCCAAAAACTTCCAGGTTC 3513
 K  M  C  D  L  T  V  * 1162
```

Ob-Ra

```
······GCACAAGGACTTAATTTCCAAAAGAGAGCGGACACTCTTTGAAGTATCTCA 2694
       A  Q  G  L  N  F  Q  KΔ R  A  D  T  L  * 894
```

Ob-Re

```
······AATGTTAACAAGTATTATATCCATGGTATGTGTACTGTGCTTTTACTTAATTAGTATGACACT 2427
       N  V  N  K  Y  Y  I  H▲ G  M  C  T  V  L  L  L  N  * 805
```

are expressed in the brain from Zucker fatty (*fa*/*fa*) rats at comparable levels to those in their lean littermates (Fig. 5B).

DISCUSSION

In the present study, we have succeeded in the isolation and sequence determination of the entire coding regions of rat Ob-Ra, Ob-Rb, and Ob-Re cDNAs. Previous studies have revealed the presence of multiple Ob-R isoforms in mice and humans (18–20, 22), which are generated by alternative mRNA splicing. The present study provides evidence that Ob-R has at least three alternatively spliced isoforms in rats. Ob-Ra and Ob-Rb are single transmembrane proteins, which differ in the C-terminal amino acid sequences. Ob-Rb has the longer intracellular domain that contains the Box 1 and Box 2 consensus sequences for interactions with JAKs and STATs (18). These observations suggest that Ob-Rb is a biologically active receptor. On the other hand, Ob-Ra has the shorter intracellular domain with the Box 1 sequence. Whether Ob-Ra is involved in signal transduction remains to be elucidated. By contrast, Ob-Re has no transmembrane domain and is a soluble form of the receptor. Analysis of the deduced amino acid sequences revealed that rat Ob-Ra, Ob-Rb, and Ob-Re are conserved in structure among species (Fig. 3).

By the use of RT-PCR with isoform-specific primers, we demonstrated that Ob-Ra, Ob-Rb, and Ob-Re mRNAs are expressed in a wide variety of rat tissues in tissue-specific manners. These results are consistent with the previous report using mouse and human tissues (18–20, 22). It has been recognized that leptin induces its satiety effect through central mechanisms (14, 15), and the hypothalamus is one of the important target sites of action of leptin in the brain. Indeed, we have recently demonstrated that direct microinjections of leptin into various hypothalamic regions reduce food intake and body weight gain in rats (Satoh et al., manuscript in preparation). It has also been shown that Ob-R is present abundantly in the choroid plexus in the brain (18, 26, 27). In the present study, significant amount of Ob-R mRNAs were present in the hypothalamus, cerebral cortex, and cerebellum. *In situ* hybridization analysis using isoform-specific oligonucleotide probes are ongoing in our laboratory to elucidate the precise distribution of each Ob-R isoform in the rat brain.

Since Ob-R mRNAs are expressed in a wide variety of peripheral tissues, it is tempting to speculate that leptin acts directly outside the brain. For instance, leptin may act directly on the female reproductive organs, in which Ob-R mRNAs are expressed (22), because treatment with leptin corrects the sterility defect in female *ob*/*ob* mice (28). On the other hand, Slieker et al. have recently reported that leptin does not affect endogenous *ob* gene expression in primary cultures of rat mature adipocytes, although treatment with leptin decreases *ob* gene expression in *ob*/*ob* mice *in vivo* (29). Further studies are necessary to elucidate the direct actions of leptin in the peripheral tissues.

In the present study, we found a missense mutation (A to C conversion at nucleotide 806) in the extracellular domain common to all the Ob-R isoforms in Zucker fatty (*fa*/*fa*) rats, thereby resulting in the Gln269Pro mutation. The mutation was found only in Zucker fatty (*fa*/*fa*) rats, but not in their lean littermates and Sprague-Dawley rats, suggesting that the

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FIG. 2. Nucleotide and deduced amino acid sequences of rat Ob-Rb, Ob-Ra, and Ob-Re isoform cDNAs. The amino acid sequences are shown in one-letter code. The translation stop codons are indicated by asterisks. The putative signal sequence and two W-S-X-W-S motifs are underlined. Transmembrane domain is doubly underlined. Box 1 and box 2 sequences are indicated by thick underlines. Sites of alternative mRNA splicing for Ob-Re, and for Ob-Rb and Ob-Ra are indicated by closed and open arrowheads, respectively. These sequence data are available from GenBank under accession Numbers D85557 to D85559.

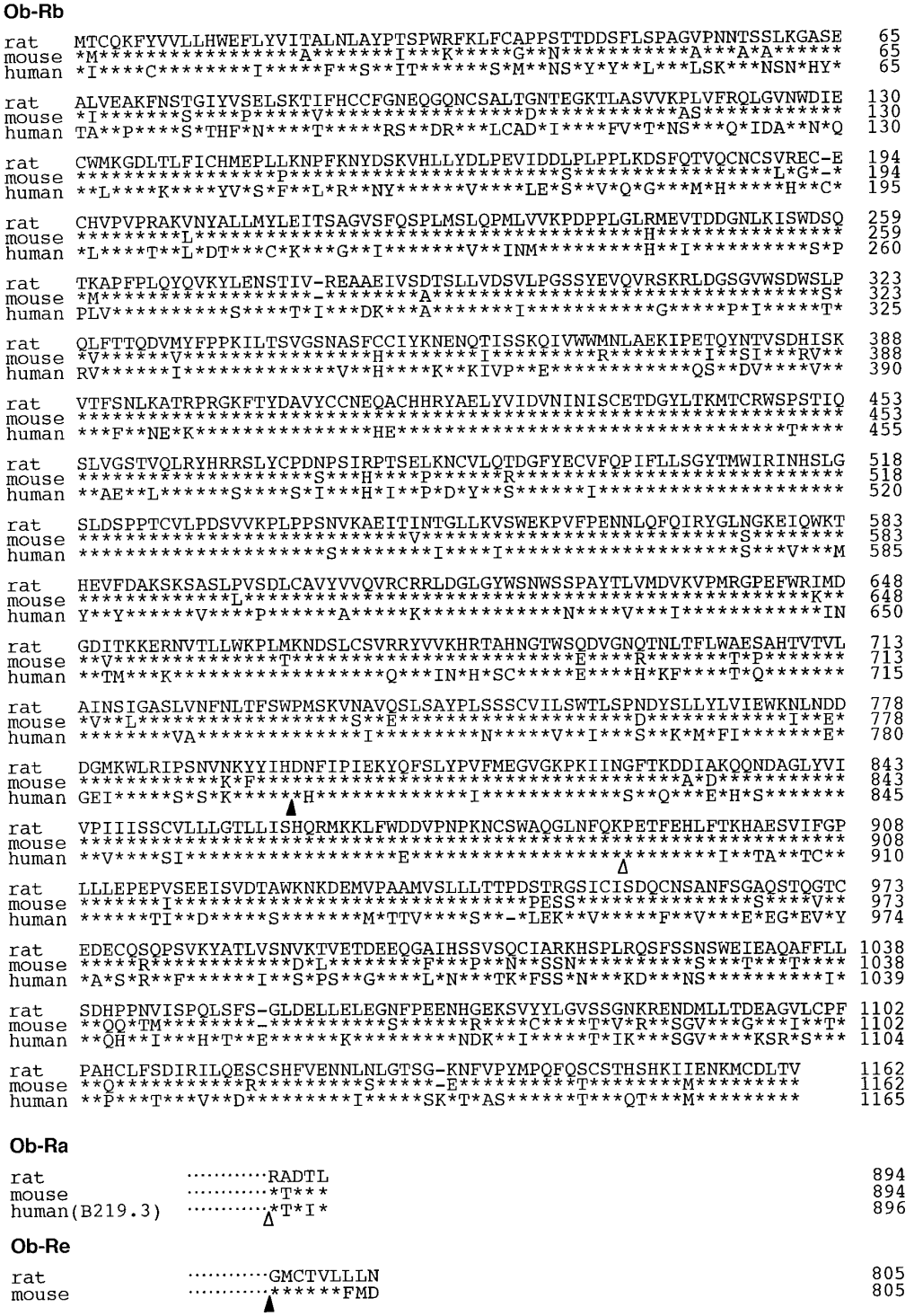

FIG. 3. Alignment of rat, mouse, and human Ob-R isoforms. Amino acid sequences are shown in one-letter code. Identical amino acids are indicated by asterisks. Amino acid sequences of mouse Ob-Ra, Ob-Rb, and Ob-Re, and human Ob-Ra and Ob-Rb are from Ref. 20, and Refs. 22 and 18, respectively.

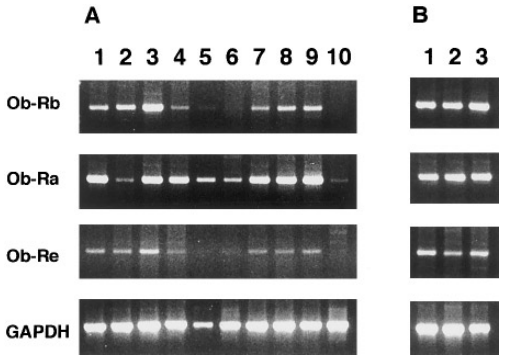

FIG. 4. (A) RT-PCR analysis of Ob-R isoform mRNAs in rats. 1, fat; 2, lung; 3, brain; 4, small intestine; 5, stomach; 6, liver; 7, kidney; 8, testis; 9, spleen; 10, heart. (B) RT-PCR analysis of Ob-Rb, Ob-Ra, and Ob-Re mRNAs in the rat hypothalamus, cerebral cortex, and cerebellum. 1, hypothalamus; 2, cerebral cortex; 3, cerebellum.

mutation is not a simple polymorphism but specific to Zucker fatty (*fa*/*fa*) rats. Furthermore, the Gln269Pro mutation introduces the Pro residue known as a β-sheet breaker (30) in a conserved region of class I cytokine receptors that lies in a β-sheet structure and is related to the fibronectin type III domain (25, 31), which should cause a dramatic structural change in Ob-R. These observations strongly suggest that the Gln269Pro mutation is the obesity-causing

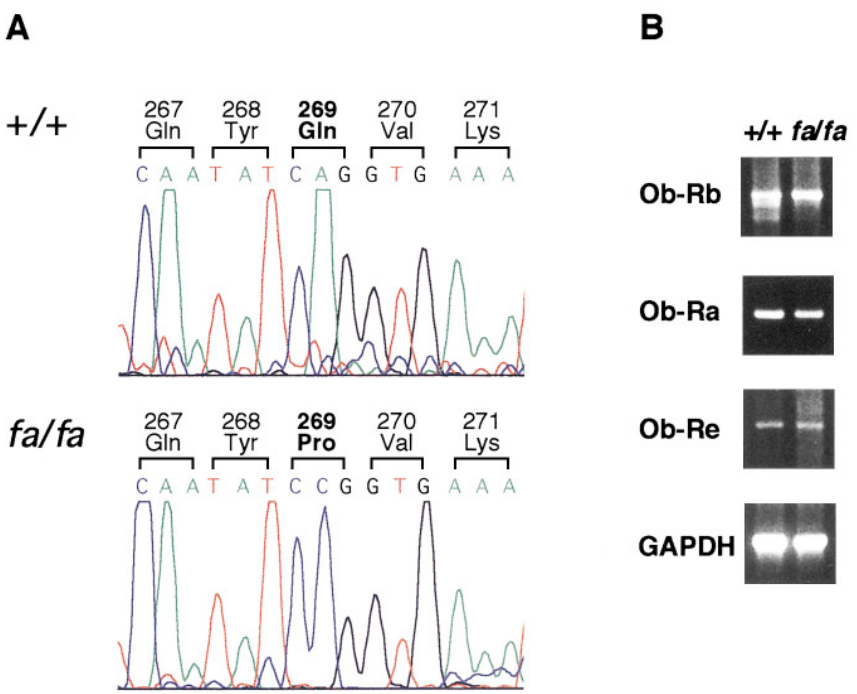

FIG. 5. (A) The Zucker fatty (*fa*/*fa*) mutation. +/+; Zucker lean rats, *fa*/*fa;* Zucker fatty (*fa*/*fa*) rats. The Zucker fatty (*fa*/*fa*) rats have an A to C change at nucleotide position 806, which causes the Gln269Pro mutation. (B) RT-PCR analysis of Ob-R isoform mRNAs in the brain from Zucker fatty (*fa*/*fa*) rats and their lean littermates (+/+). +/+; Zucker lean rats, *fa*/*fa;* Zucker fatty (*fa*/*fa*) rats.

mutation in Zucker fatty (*fa*/*fa*) rats. In the present study, Ob-R isoform mRNAs are present in the brain from Zucker fatty (*fa*/*fa*) rats. The amounts of mRNAs in Zucker fatty (*fa*/*fa*) rats were roughly equivalent to those from their lean littermates. Furthermore, ligand binding studies using ^{125}I-labeled leptin have revealed high affinity binding sites in the brain from Zucker fatty (*fa*/*fa*) rats (Ogawa et al., unpublished observations). These results suggest that the mutation does not affect Ob-R mRNA expression and/or ligand binding in Zucker fatty (*fa*/*fa*) rats. This is in a contrast to the case with *db*/*db* mice, in which Ob-Rb is missing due to a mutation that causes its abnormal transcript. Further studies are needed to elucidate the functional defect in the mutant receptor in Zucker fatty (*fa*/*fa*) rats. Characterization of the mutant receptor expressed in cultured cells *in vitro* or in transgenic animals *in vivo* will be useful to address this issue.

In conclusion, we have cloned the full-length rat Ob-Ra, Ob-Rb, and Ob-Re cDNAs and demonstrated that Ob-R isoform mRNAs are expressed in a wide variety of tissues in tissue-specific manners. We have also identified a missense mutation in the extracellular region of Ob-R. The present study has also shown that Ob-R mRNAs are expressed in the brain from Zucker fatty (*fa*/*fa*) rats. The present study should help elucidate the molecular mechanisms underlying actions of leptin.

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