Unique repetitive sequence and unexpected regulation of expression of rat endothelial receptor for oxidized low-density lipoprotein (LOX-1)

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We report the identification of a unique repetitive sequence in the rat endothelial receptor for oxidized low-density lipoprotein (LOX-1) and unexpected blood-pressure-associated regulation of its expression, a new link between lipid metabolism and blood-pressure control. A rat aorta cDNA library was constructed and screened with a probe synthesized by degenerate PCR. Rat LOX-1 cDNA encoded a protein of 364 amino acids that showed $\sim 60\,\%$ similarity to its bovine and human counterparts. The protein consisted of intracellular N-terminal, transmembrane and extracellular lectin-like domains. Rat LOX-1 was unique in having three repeats of a 46-amino-acid motif between the transmembrane and lectin-like regions. Two isoforms of mRNA were found to be generated by alternative use of two polyadenylation signals in a tissue-specific manner. The 3′-untranslated region contained multiple A + U-rich elements for rapid degradation of mRNA. Northern-blot analysis revealed that LOX-1 mRNA was expressed predominantly in the lung. Quite unexpectedly, the expression was dramatically up-regulated in the aorta in hypertensive SHR-SP/Izm rats compared with very low levels in control WKY/Izm rats, suggesting a potential role for LOX-1 in the pathogenesis of hypertension as well as atherosclerosis.

INTRODUCTION

Oxidized low-density lipoprotein (Ox-LDL) has been implicated in the pathogenesis of atherosclerosis [1–3]. It is hypothesized that Ox-LDL is trapped by a scavenger receptor on the surface of macrophages, resulting in the formation of foam cells and subsequent atherosclerotic lesions [4]. Ox-LDL is also reported to be internalized and degraded in the endothelium [5], attenuate the endothelium-dependent vasodilatory response through reduced production of nitric oxide (NO) [6,7], and induce endothelial expression of leucocyte adhesion molecules [8] and smooth-muscle growth factors [9]. These findings suggest the presence of an Ox-LDL receptor in the endothelium.

Recently, cDNA encoding a novel receptor for Ox-LDL (Lectin-like Ox-LDL receptor; LOX-1) was identified by expression cloning using bovine cultured endothelial cells [10]. LOX-1 is a membrane protein that belongs to the C-type lectin family. It is expressed in the vascular endothelium and in highly vascularized organs such as the placenta and lung. This endothelial receptor might mediate some of the above-mentioned actions of Ox-LDL in the endothelium. In the present study we performed cDNA cloning of rat LOX-1 and demonstrated several intriguing features: (1) there were triple repeats of a 46-amino-acid motif between the transmembrane and lectin-like domains, which made rat LOX-1 longer than its bovine and human counterparts; (2) the 3′-untranslated region contained multiple A + U-rich elements and polyadenylation signals; (3) the LOX-1 expression was markedly ($>$ 20-fold) up-regulated in the aorta of hypertensive rats, suggesting that the Ox-LDL/LOX-1 system has an important role in vascular biology, including regulation of lipid metabolism and blood pressure.

MATERIALS AND METHODS

Chemicals

Gigapack II Gold *in vitro* packaging kit and *Pfu* polymerase were obtained from Stratagene (La Jolla, CA, U.S.A.); mRNA purification kit and Time Saver cDNA synthesis kit were from Pharmacia (Uppsala, Sweden); restriction enzymes were from Takara (Kyoto, Japan); Sequi Therm Long-Read cycle sequencing kit was from Epicentre Technologies (Madison, WI, U.S.A.); and [α-^{32}P]dCTP was from Amersham (Amersham, Bucks., U.K.).

Animals and RNA preparation

Male stroke-prone spontaneously hypertensive rats (SHR-SP/Izm) and control Wistar Kyoto rats (WKY/Izm) ($n = 10$ for each group), 13 weeks old, were purchased from the Disease Model Cooperative Research Association (Kyoto, Japan). For the experiment involving age-dependent changes, 4-week-old male SHR-SP/Izm and WKY/Izm rats ($n = 8$ for each group) were also used. The rats were fed a normal rat chow and housed under humidity-, temperature- and light-cycle-controlled conditions. Systolic blood pressure was measured by the occlusive tail cuff and pneumatic electrosphygmomanometer (model PE-300; Narco BioSystems, Houston, TX, U.S.A.) in conscious

Abbreviations used: Ox-LDL, oxidized low-density lipoprotein; LOX-1, lectin-like Ox-LDL receptor; SHR-SP, stroke-prone spontaneously hypertensive rats; WKY, Wistar Kyoto rats; RT-PCR, reverse transcription-PCR; RACE, **r**apid **a**mplification of **c**DNA **e**nds; poly(A)$^+$, polyadenylated; PSL, photo-stimulated luminescence.

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The nucleotide sequences reported in this paper have been submitted to the DDBJ/GenBank™/EMBL Nucleotide Sequence Databases under the accession number AB005900.

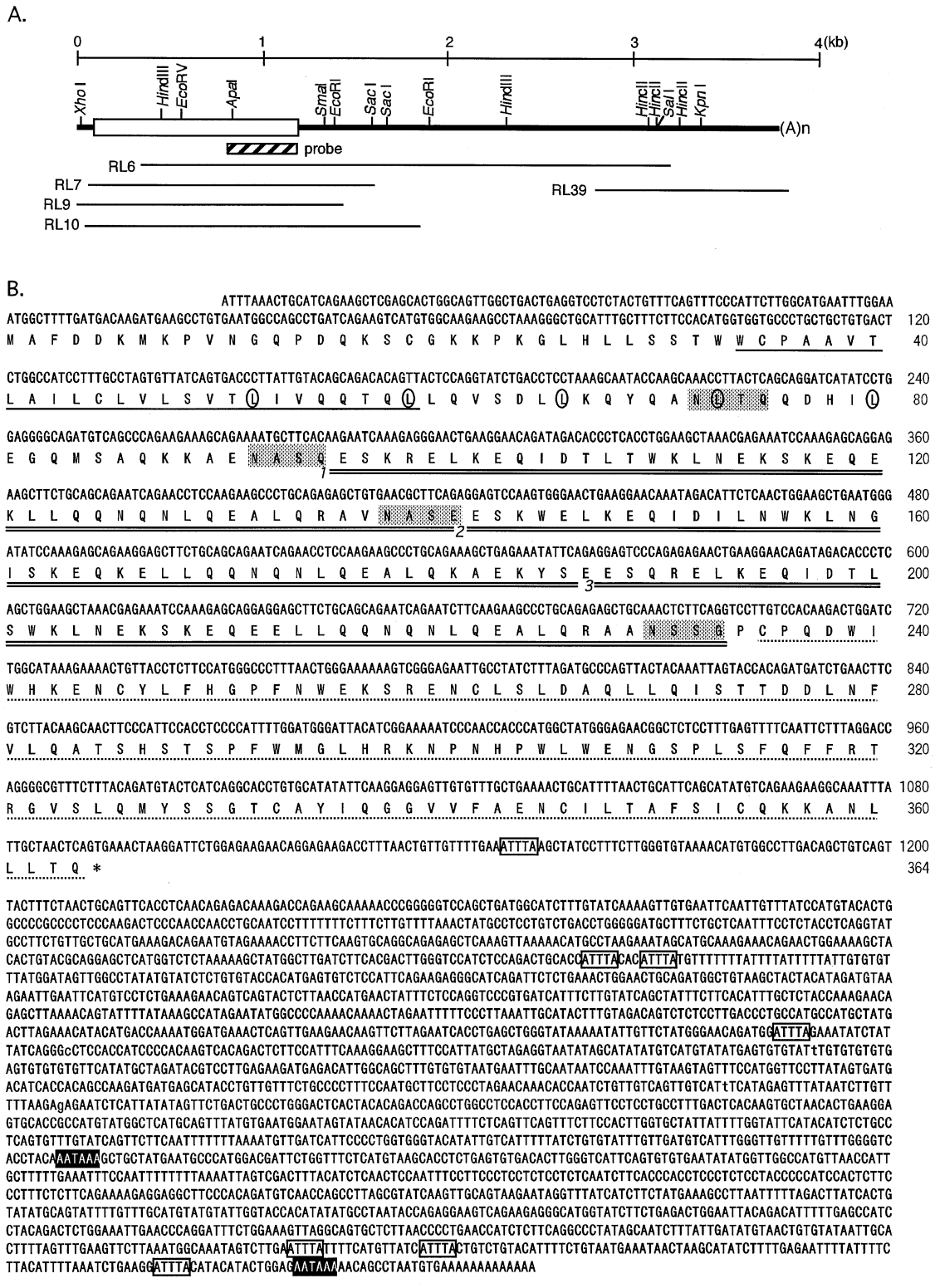

Figure 1 Cloning strategy and nucleotide and amino acid sequences of rat LOX-1

(**A**) Cloning strategy for rat LOX-1 cDNA. The scheme for rat LOX-1 cDNA is shown on the top with restriction-enzyme sites. The open reading frame is shown by an open box. The probe used for screening the rat aorta cDNA library is indicated by the hatched box. The isolated clones are indicated by bars (RLs 6, 7, 9 and 10). The most 3′-side portion was determined by 3′-RACE PCR (RL 39). (**B**) The nucleotide and deduced amino acid sequences. Position numbers are shown on the right. The first nucleotide of the open reading frame is numbered as 1. The putative transmembrane domain is underlined. The potential N-glycosylation sites are shaded. Three repetitive regions are double underlined. The region similar to C-type lectin is shown by a dotted line. Two polyadenylation signals (AATAAA) are indicated by white letters on a black background. The sequence motifs for rapid degradation of mRNA (ATTTA) are boxed. A leucine zipper-like motif is present at the extracellular juxtamembrane domain, which contains a leucine at every seventh residue: Leu^{52}, Leu^{59}, Leu^{66}, Leu^{73} and Leu^{80} (indicated by a circle).

prewarmed rats. On the following day the rats were killed by decapitation. Organs were dissected, immediately frozen in liquid nitrogen, and stored at −80 °C until use. Total RNA was extracted by the acid guanidinium thiocyanate/phenol/chloroform method [11]. Polyadenylated [poly(A)$^+$] RNA was purified using an mRNA purification kit.

Probe preparation

The probe for rat LOX-1 was synthesized by reverse transcription-PCR (RT-PCR). A 10 μg portion of total RNA from the aorta of the SHR-SP/Izm rat strain was reverse-transcribed to cDNA with Moloney-murine-leukaemia-virus reverse transcriptase and oligo(dT)$_{17}$ primer. The resultant cDNA (1/20 of total) was subjected to PCR with *Pfu* polymerase using degenerate primers: 5′-CTGGMTCTGGCATGRAGAAA-3′ and 5′-YGCCTTCTTYTGACATATACTG-3′. The amplification was carried out for 35 cycles by repeating 95 °C for 1 min, 43 °C for 1 min, and 75 °C for 3 min.

Construction and screening of the cDNA library

Complementary DNA was synthesized from the SHR-SP/Izm rat aorta poly(A)$^+$ RNA (5 μg) with random primers using a Time Saver cDNA synthesis kit. The cDNA was ligated to the λZAP II phage vector and packaged using a Gigapack II Gold *in vitro* packaging kit. Approx. 10^6 recombinant phages were obtained. The phages were plated at a density of 30000 plaques/15 cm plate, and two replica nitrocellulose filters were prepared from each plate. The PCR-generated probe for rat LOX-1 was labelled with [α-^{32}P]dCTP by the random-primer-labelling method. The filters were prehybridized in a solution containing 50 % formamide, 6 × SSC (1 × SSC is 150 mM NaCl/15 mM sodium citrate, pH 7.0), 5 × Denhardt's solution [1 × Denhardt's solution is 0.02 % (w/v) poly(vinylpyrrolidone)/0.02 % (w/v) Ficoll/0.02 % (w/v) BSA], 100 μg/ml sonicated herring sperm DNA, and 0.1 % (w/v) SDS at 37 °C for 2 h and hybridized to the ^{32}P-labelled probe (10^6 c.p.m./ml) at 42 °C for 16 h. The hybridized filters were washed twice in 2 × SSC and 0.1 % (w/v) SDS at room temperature for 5 min, twice in 0.1 × SSC and 0.1 % (w/v) SDS at 60 °C for 1 h and then exposed to Kodak X-OMAT AR5 film with an intensifying screen at −80 °C for 48 h. Plaques that gave positive signals on both replicas were selected and purified. The second and the third rounds of screening were carried out under the same conditions to isolate the positive clones. Positive phages were converted into plasmid cDNA by rescue excision using helper phage R408, and then analysed.

3′-RACE PCR

Poly(A)$^+$ RNA (1 μg) from the lung of the SHR-SP/Izm rat strain was reverse-transcribed to cDNA using the oligo(dT)-adapter, 5′-GACTCGAGTCGACATCGATTTTTTTTTTTT-TTTTT-3′. The resultant cDNA was amplified by PCR using a specific primer (5′-GTTTCTTCCACTTGGTGCTA-3′) and the adapter primer (5′-GACTCGAGTCGACATCG-3′) [12]. The products were subcloned to pBluescript II and sequenced.

Sequence analysis

Nucleotide sequences were determined on both strands by the dideoxynucleotide chain-termination method [13] using a Sequi Therm Long-Read cycle sequencing kit and an automated laser fluorescent DNA sequencer (LI-COR, Lincoln, NE, U.S.A.). The sequences were analysed using the GENETYX-MAC software (Software Development, Tokyo, Japan).

Northern-blot analysis

Poly(A)$^+$ RNA (2 μg) from various organs of WKY/Izm and SHR-SP/Izm rats was fractionated on a formaldehyde-denatured 1.2 %-agarose gel and transferred to a nylon membrane filter. After prehybridization, the filter was hybridized with the ^{32}P-labelled probe (10^6 c.p.m./ml) in a solution containing 50 % formamide at 42 °C for 16 h. The membrane was washed twice in 2 × SSC containing 0.1 % (w/v) SDS at room temperature, followed by washes in 0.2 × SSC containing 0.1 % (w/v) SDS at 60 °C for 1 h. The filter was exposed to Kodak X-OMAT AR5 film with an intensifying screen at −80 °C. For quantitative analysis, the Northern filter was exposed to an imaging plate, and radioactivity of the bands was quantified as photo-stimulated luminescence (PSL) with a Bioimage Analyzer (model BAS 2000; Fuji Film, Tokyo, Japan). PSL value for LOX-1 was standardized with that of glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Probe preparation

We synthesized a pair of degenerate PCR primers based on sequences highly conserved between bovine and human LOX-1 cDNA. RNA from the aorta of the SHR-SP/Izm rat strain was subjected to RT-PCR using the degenerate primers to amplify a rat LOX-1 cDNA fragment. We obtained a PCR product of the expected size (361 bp). The nucleotide sequence showed 76 % similarity to bovine and 78 % to human LOX-1 cDNA [10].

Isolation and sequence analysis of rat LOX-1 cDNA clones

An SHR-SP/Izm rat aorta cDNA library was constructed and screened with the PCR-generated rat LOX-1 probe. Figure 1(A) depicts the cloning strategy. Four positive clones (RLs 6, 7, 9 and 10 in Figure 1A) were isolated from 10^6 phages. The 3′ end was determined by 3′-RACE PCR (RL 39 in Figure 1A). The nucleotide and deduced amino acid sequences are shown in Figure 1(B). The combined cDNA contained 91 nucleotides of the 5′-untranslated region, 1095 nucleotides of coding region, 2564 nucleotides of the 3′-untranslated region, and a poly(A) tail of 13 bases. The open reading frame encodes a protein of 364 amino acid residues with a calculated M_r of 41890. The 3′-untranslated region contained two polyadenylation signals (AATAAA) and seven consensus motifs for rapid mRNA degradation (ATTTA, A + U-rich element).

Comparative analysis of LOX-1 protein

The amino acid sequence for LOX-1 was compared among rat, bovine, and human LOX-1 proteins (Figure 2). Rat LOX-1 protein was approx. 100 amino acids longer than bovine (270 amino acids) and human (273 amino acids) LOX-1 proteins. As observed in the bovine and human receptors, rat LOX-1 had a C-type lectin-like domain in the extracellular region. The cytoplasmic (amino acid residues 1–33), a hydrophobic transmembrane (residues 34–59) and extracellular lectin-like domains (residues 235–364) of rat LOX-1 were 41, 62 and 67 % similar to

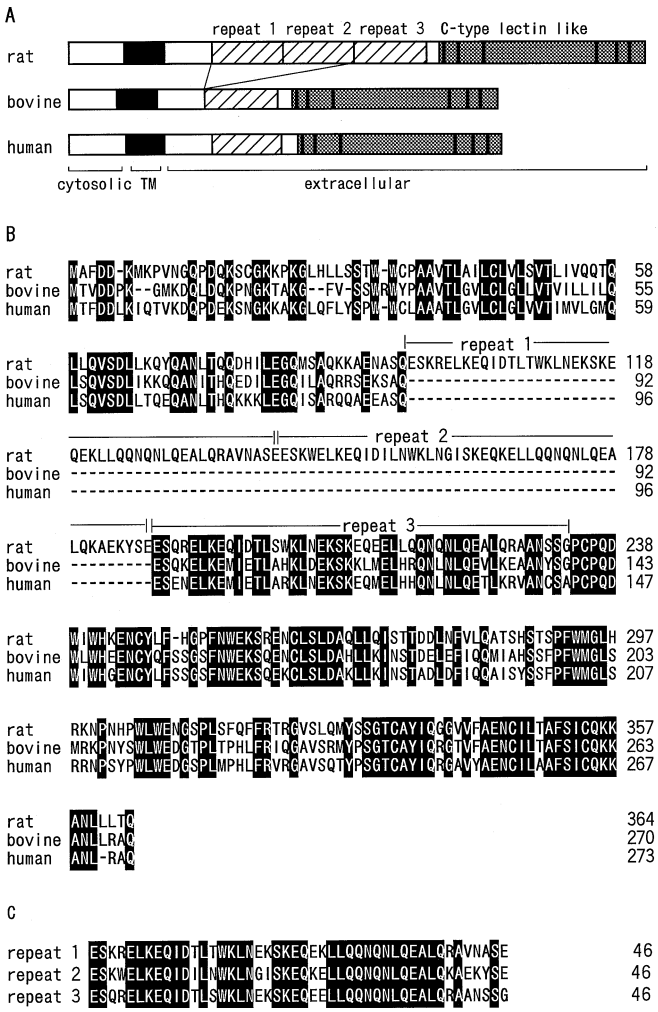

Figure 2 Comparison of rat, bovine and human LOX-1 and the presence of a triple repeat in the rat sequence

(**A**) Schematic representation of structures of rat, bovine, and human LOX-1. Solid boxes represent the transmembrane (TM) domain. Grey boxes represent the C-type lectin-like domain. Vertical bold lines indicate cysteine residues in the C-type lectin-like domain. Hatched boxes represent the 46-amino-acid repeat unit. Note that only rat LOX-1 contains the triple repeat. (**B**) Alignment of the amino acid sequence of rat LOX-1 with those of bovine and human LOX-1. Identical amino acids among the three species are shown by white letters on a black background. Gaps are indicated by bars to achieve maximum matching. Positions of repeats 1–3 are shown by an overline. (**C**) Alignment of repeats 1–3 in rat LOX-1. Identical amino acids among the three are shown by white letters on a black background.

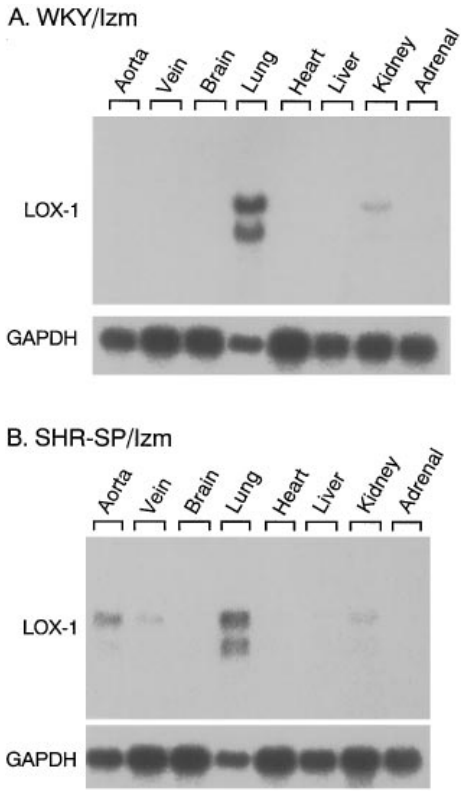

Figure 3 Tissue distribution of rat LOX-1 mRNA and marked alteration in hypertensive rats

Gene expression of LOX-1 was examined in various rat organs from 13-week-old male WKY/Izm rats (**A**) and age-matched SHR-SP/Izm rats (**B**) using Northern blotting. Systolic arterial pressure is shown in Figure 4(D). Poly(A)$^+$ RNA (2 μg) from the indicated organs was fractionated on a denatured 1.2%-agarose gel and hybridized with the ^{32}P-labelled LOX-1 probe. Two LOX-1 mRNA species are due to the alternative termination of transcription. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used as a loading control. The data shown are representative of a typical experiment repeated three times with similar results.

the corresponding portions of bovine LOX-1, and 59, 58 and 71% to those of human LOX-1 (Figure 2B). The six cysteine residues in the lectin-like domain were conserved among rat, bovine and human LOX-1 (vertical bold lines in Figure 2A). The most striking feature of the rat LOX-1 protein was the presence of three repeats (residues 96–141, 142–187 and 188–233) between the transmembrane and lectin-like domains (Figure 2). Bovine and human LOX-1 contain only one repeating unit. Each repeat consisted of 46 amino acid residues rich in Glu (E), Gln (Q), Leu (L), and Lys (K), which were weakly similar to the repeating units in the rod-like portion of the myosin II heavy chain (P08799). Repeats 1–3 in rat LOX-1 were 86–91 similar to one another (Figure 2C). Repeat 3 corresponded to the bovine (70% similarity) and human (77%) motifs. The triplication made rat LOX-1 longer than that of bovine and human.

Tissue distribution of rat LOX-1 mRNA

Gene expression of LOX-1 was examined in various organs of 13-week-old WKY/Izm and SHR-SP/Izm rats using Northern-blot analysis (Figure 3). The PCR-generated rat LOX-1 probe hybridized to two transcripts. In control WKY/Izm rats, LOX-1 was expressed predominantly in the lung and at a much lower level in the kidney. In hypertensive SHR-SP/Izm rats, on the other hand, LOX-1 expression was dramatically up-regulated in the aorta ($\sim$20-fold) and vein (inferior vena cava) (5-fold) compared with those in WKY/Izm rats. The expression in the lung and kidney did not differ between SHR-SP/Izm and WKY/Izm rats. We performed another series of Northern-blot analyses using RNA from various organs of WKY/Izm and SHR-SP/Izm rats and confirmed the reproducibility of the result.

Comparison of LOX-1 expression among SHR-SP/Izm and WKY/Izm rats at 4 and 13 weeks of age

The LOX-1 mRNA levels in the aorta were compared among pre-hypertensive and hypertensive SHR-SP/Izm rats and age-

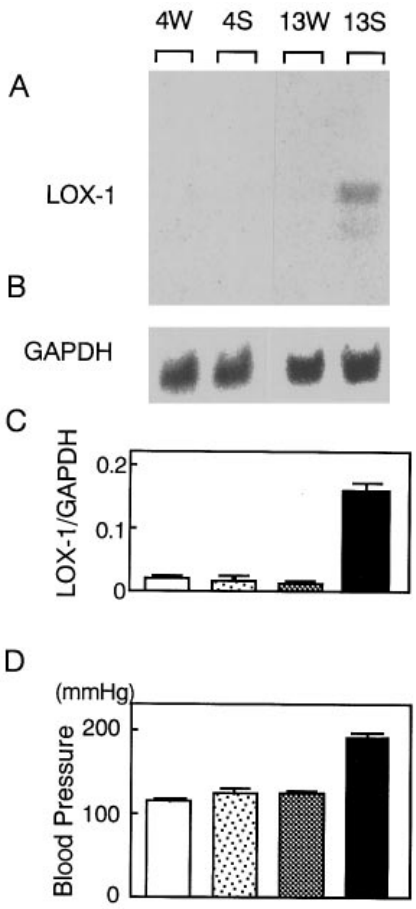

Figure 4 Comparison of aortic LOX-1 mRNA expression among young and adult SHR-SP/Izm and WKY/Izm rats

Gene expression of LOX-1 was examined as described in Figure 3. (**A**) Representative autoradiograph of 4-week-old WKY/Izm rats (4W), 4-week-old SHR-SP/Izm rats (4S), 13-week-old WKY/Izm rats (13W), and 13-week-old SHR-SP/Izm rats (13S) are shown. (**B**) A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used as a loading control. (**C**) Bar graph demonstrates the result of quantitative analysis. The radioactivity of the bands ($n = 4$ for each group) was quantified by BAS 2000, and standardized by the GAPDH expression level. Data are means $\pm$ SEM. (**D**) Systolic arterial pressure of 4- and 13-week-old WKY/Izm and SHR-SP/Izm rats ($n = 5$ for each group) measured by the tail-cuff method are shown.

matched WKY/Izm rats (Figure 4). In pre-hypertensive 4-week-old SHR-SP/Izm rats, the expression was very low and did not differ from that in age-matched WKY/Izm rats. On the other hand, in 13-week-old SHR-SP/Izm rats with established hypertension, LOX-1 expression was markedly enhanced compared with the low levels in age-matched normotensive WKY/Izm rats.

Two isoforms of rat LOX-1 mRNA

As shown in Figure 3, two bands (4.0 and 3.4 kb) were observed using Northern-blot analysis. Sequence analysis revealed that the 3′-untranslated region of rat LOX-1 contained two polyadenylation signals (AATAAA) located 752 bp apart (white letters on black background in Figure 1B). To examine whether the two isoforms were generated by alternative use of polyadenylation signals or by alternative splicing, we amplified several portions of LOX-1 cDNA using PCR with lung poly(A)$^+$ RNA. Amplification of the 3′ ends by 3′-RACE PCR yielded two cDNA fragments. The longer cDNA had a poly(A) tail 14 bp downstream of the second polyadenylation signal. The shorter cDNA had a truncated 3′ end. Its poly(A) tail was located 16 bp downstream of the first polyadenylation signal. On the other hand, amplification of the entire coding region yielded only one band (results not shown). These results suggest that the two transcripts are generated from a single gene by alternative choice of polyadenylation signals.

The radioactivity of the two bands was compared in various organs. The relative abundance of the two transcripts varied in a tissue-specific manner: The ratio of the longer transcript to the shorter one was 3:2 in the lung, whereas it was $\sim$ 6:1 in the aorta, vein and kidney.

DISCUSSION

In the present study we isolated and characterized rat LOX-1 cDNA and demonstrated that the rat receptor has, unlike its bovine and human counterparts, a triple repeat structure (repeats 1–3) consisting of a 46-amino-acid motif in the extracellular domain. Bovine and human LOX-1 have only one such motif. Owing to the presence of the two additional motifs, rat LOX-1 is approx. 100 amino acids longer than bovine and human LOX-1. The motif identified by the present study might have important functional roles and serve as a target for deletion analysis. Except for the extra portions, rat LOX-1 was 62 % similar to the bovine and 67 similar to the human receptors.

LOX-1 is an Ox-LDL receptor expressed on the endothelium [10]. It consists of intracellular N-terminal, transmembrane, 46-amino-acid repetitive, and C-type lectin-like domains with conserved cysteine residues. A scavenger receptor is another Ox-LDL receptor which is expressed in macrophages [4]. Although a homology search did not reveal significant similarity between the scavenger receptor and LOX-1, the two seem to be somewhat similar in their structural organization; the macrophage protein consists of intracellular N-terminal, transmembrane, α-helical coiled-coil with N-glycosylation sites, collagen-like ligand-binding, and C-terminal cysteine-rich domains. Moreover, the repeating units in LOX-1 showed a weak similarity to the rod-like tail of the myosin II heavy chain (P08799). The rod-shaped tail sequence of the myosin II heavy chain is highly repetitive, and the periodicity of hydrophobic and charged residues dictates the α-helical coiled-coil structure [14]. The significance of the triple-repeat structure in rat LOX-1 is unclear at present, but further studies such as site-directed mutagenesis and deletion analyses will clarify the structure–function relationship. Repeats 1–3 in rat LOX-1 were $\sim$ 90 % identical with one another. The similarity among different species was much lower ($\sim$ 75 %). There is, therefore, a possibility that a 46-amino-acid unit is encoded by a single exon, which was triplicated and diversified after speciation of rat, bovine and human.

Another prominent feature of rat LOX-1 is the marked enhancement of gene expression in the vessels (especially in the aorta) of the hypertensive rat model (Figure 3). The up-regulation was tissue-specific, and no apparent increase was observed in the lung, kidney and other organs of SHR-SP/Izm rats compared with those of WKY/Izm rats. Moreover, the up-regulation in the aorta of SHR-SP/Izm rats was not observed at the pre-hypertensive stage, but only after the development of hypertension, suggesting that LOX-1 up-regulation in the aorta of SHR-SP/Izm rats is related to blood-pressure elevation (Figure 4). LOX-1 has been considered as an endothelial receptor for processing modified LDL, such as Ox-LDL, and has recently been cloned by expression cloning using cultured endothelial cells [10]. Recent studies, however, demonstrated that Ox-LDL, a native ligand for LOX-1, has several effects on endothelial cells

other than the clearance of Ox-LDL from plasma. For example, it reduces endothelial production of NO [6,7] and induces endothelial expression of leukocyte adhesion molecules [8] and smooth-muscle growth factors [9], suggesting that the Ox-LDL/LOX-1 system has a variety of roles in vascular physiology. In this context, our demonstration that the aortic levels of LOX-1 are markedly elevated in the hypertensive SHR-SP/Izm rats (Figure 3) is very interesting and opens a new avenue for hypertension research. Although the link between the elevated levels of LOX-1 and high blood pressure should be addressed in future studies, one explanation could be that the elevated expression of LOX-1 enhances the attenuating effect of Ox-LDL on the endothelium-dependent vasodilatory response through NO inhibition.

The unexpected variety of roles of the Ox-LDL/LOX-1 system suggest versatile co-ordination of the components. The characteristic motifs identified in the 3′-untranslated region of LOX-1 mRNA, namely multiple signals for rapid degradation of mRNA (A + U-rich elements) and two polyadenylation signals (AATAAA) that generate short and long transcripts (Figure 1B), are expected to be used in such versatile regulation.

In conclusion, we determined the amino acid sequence of a rat endothelial receptor for Ox-LDL (LOX-1) and its tissue distribution in a hypertensive rat model and found that: (1) rat LOX-1 has a triple repeat consisting of 46 amino acids; (2) the rat LOX-1 message has, in its 3′ region, multiple regulatory elements such as A + U-rich elements that are responsible for rapid turnover of the message and AATAAA that is responsible for generation of multiple mRNA species of different sizes; and (3) the message levels are markedly elevated in the aorta of hypertensive SHR-SP/Izm rats (> 20-fold). The identification of the repetitive sequence in the extracellular domain of LOX-1 will facilitate the analysis of the structure–function relationship of the receptor, and the demonstration of its greatly enhanced expression in the aorta of hypertensive rats suggests a strong, but quite unexpected, link between the metabolism of modified LDL and the control of blood pressure, opening a new field of research in vascular biochemistry, physiology and pathophysiology.

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REFERENCES

1 Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H. and Kawai, C. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 5928–5931
2 Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C. and Witztum, J. L. (1989) N. Engl. J. Med. **320**, 915–924
3 Witztum, J. L. and Steinberg, D. (1991) J. Clin. Invest. **88**, 1785–1792
4 Kodama, T., Freeman, M., Rohrer, L., Zabrecky, J., Matsudaira, P. and Krieger, M. (1990) Nature (London) **343**, 531–535
5 Pitas, R. E., Boyles, J., Mahley, R. W. and Bissel, D. M. (1985) J. Cell Biol. **100**, 103–117
6 Kugiyama, K., Kerns, S. A., Morrisett, J. D., Roberts, R. and Henry, P. D. (1990) Nature (London) **344**, 160–162
7 Yokoyama, M., Hirata, K., Miyake, R., Akita, H., Ishikawa, Y. and Fukuzaki, H. (1990) Biochem. Biophys. Res. Commun. **168**, 301–308
8 Kume, N., Cybulsky, M. I. and Gimbrone, Jr., M. A. (1992) J. Clin. Invest. **90**, 1138–1144
9 Ross, R. (1993) Nature (London) **362**, 801–809
10 Sawamura, T., Kume, N., Aoyama, T., Moriwaki, H., Hoshikawa, H., Aiba, Y., Tanaka, T., Miwa, S., Katsura, Y., Kita, T. and Masaki, T. (1997) Nature (London) **386**, 73–77
11 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
12 Frohman, M. A., Dush, M. K. and Martin, G. R. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 8998–9002
13 Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 5463–5467
14 Warrick, H. M., De Lozanne, A., Leinwand, L. A. and Spudich, J. A. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 9433–9437