FULL PAPER *Ethology*

Molecular Cloning of Canine Monoamine Oxidase Subtypes A (MAOA) and B (MAOB) cDNAs and Their Expression in the Brain

Chie HASHIZUME[1)], Masatoshi SUZUKI[2)], Koji MASUDA[1)], Yukihide MOMOZAWA[1)], Takefumi KIKUSUI[1)], Yukari TAKEUCHI[1)]* and Yuji MORI[1)]

[1)]Laboratories of Veterinary Ethology and [2)]Veterinary Physiology, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan

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ABSTRACT. The role of monoamine oxidase has been shown to be related to some behavioral changes including aggression and cognitive dysfunction. In order to demonstrate the basic expression patterns of monoamine oxidase in the canine brain, we determined the full-length nucleotide sequences of cDNA for canine monoamine oxidase type A (MAOA) and type B (MAOB) genes that were isolated from the canine brain cDNA library. Oligonucleotide primers for PCR were constructed based on the conserved sequences reported thus far for other mammalian species. The nucleotide sequences had open reading frames of 1584 and 1563 bp for MAOA and MAOB, respectively. Both of these genes showed relatively high homology with other species in both nucleotide (> 81%) and deduced amino acid (> 85%) sequences. In Northern blot analyses MAOA mRNA was expressed broadly in various parts of the canine brain, whereas MAOB mRNA was found only in specific brain regions, such as the hypothalamus, hippocampus, brain stem and olfactory bulb. These results suggest that MAOA and MAOB mRNAs have subtype-specific expression patterns in the canine brain.

KEY WORDS: canine, cDNA, monoamine oxidase, Northern blot analysis, temperament.

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Monoamine Oxidase (MAO) is an enzyme that is localized on the outer membrane of mitochondria and that catalyzes oxidative deamination of various biogenic amines in the brain as well as in peripheral tissues [17]. On the basis of substrate selectivity and sensitivity to inhibitors, it has been postulated that there are two forms of MAOs: MAOA and MAOB [9, 11]. In human study, MAOA has a higher affinity than MAOB for substrates such as serotonin (5-HT), norepinephrine (NE) and dopamine (DA), and its irreversible inhibitor is clorgyline. On the other hand, MAOB has an affinity with dopamine (DA), phenylethylamine (PEA) and benzylamine, and its inhibitor is deprenyl. Both types of MAO consist of a flavin-containing active site [14], and the cofactor flavin-adenine dinucleotide (FAD) is revealed to be covalently linked to the cysteine residue of the same pentapeptide (Ser-Gly-Gly-Cys-Tyr) for both MAOA and MAOB in human study. The amino acid sequences of the pentapeptide are found in some species; human, bovine, and rat as far reported [2, 5, 9, 14].

Recent studies have implicated MAO in several behavioral changes. For instance, MAOA knockout mice showed increased aggression in adulthood [5]. Brunner *et al.* [4] described that a nonsense mutation in the human MAOA gene was associated with an impulsive aggression. These results suggested an association between MAOA deficiency and abnormal behavior. Regarding MAOB, altered enzymatic activity levels in platelets were described in people with impulsivity [10], and administration of l-deprenyl, a selective inhibitor of MAOB, was found to have some beneficial effects on spatial short-term memory in aged dogs [16] and on the mental performance of aged dogs that had been exhibiting cognitive dysfunction [13]. Therefore, brain MAO is thought to play an important role in cognitive function and thus is probably involved in some forms of psychiatric disorders as well as aggressive behavior. However, species differ remarkably in tissue distribution patterns and in the spectra of targeting substrates for deamination between, for example, humans and rodents. At this moment, no data are available on the tissue distribution of MAO mRNA in canines. In order to clarify the species-specific tissue distribution pattern in the canine brain, it is necessary to isolate and characterize cDNA clones encoding both MAOA and MAOB genes. In the present study, we therefore attempted to clone canine MAOA and MAOB cDNAs from the canine brain cDNA library and to determine their nucleotide sequences. Northern blot analysis was then carried out to localize the respective expressions of MAOA and MAOB mRNAs in the brain.

* CORRESPONDENCE TO: TAKEUCHI, Y., Laboratory of Veterinary Ethology, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.

MATERIALS AND METHODS

Preparation of total RNA and synthesis of cDNAs: Canine tissues for RNA isolation were obtained from two healthy dogs (female, Beagle) sacrificed by an excess dose of anesthetics. The experimental procedures followed the guidelines of "Policies Governing the Use of Live Vertebrate Animals" by The University of Tokyo, based on "The Public Health Service Policy on Humane Care and Use of Laboratory Animals" by Awardee institutions (revised in 1985) and "The National Institutes of Health Guide for the Care and Use of Laboratory Animals" (revised in 1985). Total RNA was purified with TRIzol® reagent (Gibco BRL,

U.S.A.) from various regions of the canine brain. For RT-PCR, a first-strand cDNA was synthesized from 1 μg of the isolated RNA as a template using oligo $d(T)_{16}$ with GeneAmp® RNA PCR Kit components (Perkin-Elmer, U.S.A.) according to the manufacturer's instructions.

Sequencing full-length cDNA of canine MAOA and MAOB: In order to amplify the coding regions of canine MAOA and MAOB cDNAs, oligonucleotide primers for PCR were designed based on the conserved sequences of human [2], bovine [14] and rat [9] MAOA genes: the sequence of the forward primer was 5'-GGACTATCTGCTGCCAAACT -3', and that of the reverse primer was 5'-TGAGGCTCAGAGGTCACATT -3'. The primers for MAOB genes were also designed from the conserved sequences of human [2] and rat [8] MAOB genes. The sequence of the forward primer was 5'- CTCTGGGCATGAAGATTCAC -3', and that of the reverse primer was 5'-TTCATCCTCTGGAATCTTCC -3'. The PCR was performed in a 100 μl reaction volume with 4 μl of the cDNA prepared above. The PCR solution was composed of 200 μM dNTPs, 10 μl 10 × Ex Taq Buffer (Takara, Japan), 0.2 μM of each primer and 2.5 units TaKaRa Ex Taq (Takara, Japan). After the initial denaturation at 95°C for 5 min, PCR was performed by 30 successive cycles of 94°C for 1 min, 55°C 1 min and 72°C 1 min. Chain elongation at 72°C was extended to 10 min after the final cycle.

The entire PCR product was then separated in 2.0% agarose gel, and the band of the expected size was excised and purified using the QIAquick gel extraction kit (Qiagen, U.S.A.). Then, DNA sequencing was performed by the Big-Dye Terminator Cycle Sequencing FS Ready Reaction Kit using the ABI PRISM 377 DNA sequencer (Perkin-Elmer, U.S.A.).

The PCR primers based on the obtained sequences were designed to screen the Beagle brain cDNA library (generously provided by Dr. Minoru Suzuki). For the 5' region, the forward primer was constructed based on the vector plasmid (pME18SFL3) sequence, and the reverse primer was based on the canine MAOA or MAOB cDNA fragment sequence. On the other hand, for the 3' region, the forward primer was designed based on the canine MAOA or MAOB cDNA fragment, and the reverse primer was based on the vector plasmid (pME18SFL3) sequence. The PCR was performed using these primers with the canine cDNA library as a template. The PCR products were then processed for direct sequencing as described above.

Northern blot analysis: Three micrograms of total RNA extracted from each of various regions of the canine brain (i.e., the cerebellum, hypothalamus, hippocampus, amygdala, frontal cortex, temporal cortex, brain stem, corpus striatum and olfactory bulb) was separated by electrophoresis in 1% agarose MOPS-formaldehyde gels and transferred to a nylon membrane (Biodyne, Pall Biosupport, Japan). After blotting, each membrane was dried at 80°C for 30 min and then prehybridized at 65°C for 90 min in the following hybridization solution: 5 × standard saline citrate (SSC), 50% formamide, 50 mM sodium phosphate buffer, 7% SDS, 2% DIG-blocking reagent (Roche Diagnostics KK, Japan), 0.1% N-laurylsarcosine and 0.05 mg/m*l* salmon sperm DNA in diethylpyrocarbonate (DEPC)-treated water. Hybridization was then performed at 65°C for 16 hr by adding a DIG-11-UTP-labeled antisense cRNA probe for MAOA, MAOB or β-actin, independently. After hybridization, each membrane was washed in 2 × SSC/0.1% SDS at 65°C for 5 min twice and then washed in 0.5 × SSC/0.1% SDS at 65°C for 20 min twice. Thereafter, the membrane was incubated with an anti-DIG alkaline phosphatase-conjugated antibody (Roche Diagnostics KK, Japan) and further processed with CDP-Star (Roche Diagnostics KK, Japan) for chemiluminescence detection of DIG as recommended by the manufacturer. The signal was developed on X-ray film (Hyperfilm; ECL, Japan) for 15 min.

RESULTS

Canine MAOA and MAOB cDNA and predicted amino acid sequences: Figure 1 shows the nucleotide sequence and deduced amino acid sequence for the entire MAOA cDNA (GenBank database accession: AB038563). The canine MAOA coding region consisted of 1584 nucleotides and 527 deduced amino acids (calculated molecular mass of 60.0 kDa), and had a sequence for a putative FAD-binding site. Figure 2 shows the nucleotide and deduced amino acid sequences for the entire MAOB cDNA (GenBank database accession: AB070958). The canine MAOB coding region consisted of 1563 nucleotides and 520 deduced amino acids (calculated molecular mass of 58.4 kDa), and it had a sequence for the putative FAD-binding site.

The canine MAOA sequence had identity of greater than 81% with other species (human, cattle and rat) [6, 12, 15] at the nucleotide level and homology of greater than 85% for the amino acid sequence. The canine MAOB sequence also had a high level of identity, greater than 85%, with other species (human and rat) [6, 8] at the nucleotide level and homology of greater than 88% for the amino acid sequence (Table 1).

Northern blot analysis of canine MAOA and MAOB cDNA: The results of Northern blot analyses conducted in two animals were consistent. Northern blot analysis of MAOA mRNA revealed a single major transcript at 4.8 kb in the RNA samples from most of the brain tissues (Fig. 3). Although the concentrations of these transcripts were found to be about equal in amount, the relative concentrations of the transcripts from the cerebellum and the olfactory bulb were low. The expression pattern of the MAOB mRNA in the canine brain was quite different from that of MAOA. The transcript in the hypothalamus had the highest level of MAOB mRNA, and relatively large amounts of mRNA were detected in the hippocampus, brain stem and the olfactory bulb. Small amounts of mRNA were detected in the amygdala, frontal cortex, temporal cortex and corpus striatum. However, under this hybridization condition, any amount of mRNA MAOB could not have been detected in the cerebellum. The hybridization of β-actin showed that

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                                                    1    TGGCCTACTGGAGTCTCTACCAGACC      26

ATGGCGAGTAGAGAGAAGACGAGTATCGAGGGCCACATGTTTGACGTAGTCGTGATAGGAGGCGGCATCTCAGGATTGTCTGCTGCCAAA     116
MetAlaSerArgGluLysThrSerIleGluGlyHisMetPheAspValValValIleGlyGlyGlyIleSerGlyLeuSerAlaAlaLys      30

CTCTTAGCCGAACATGAAGTTGATGTCTTAGTTTTAGAAGCACGAGATAGAGTTGGAGGAAGAACATACACCGTGAGGAATGAACATGTC     206
LeuLeuAlaGluHisGluValAspValLeuValLeuGluAlaArgAspArgValGlyGlyArgThrTyrThrValArgAsnGluHisVal      60

GATTACGTAGATGTTGGTGGGGCTTATGTAGGACCAACTCAGAACAGAATCTTACGACTATCTAAGGAGCTGGGTCTAGAGACTTACAAA     296
AspTyrValAspValGlyGlyAlaTyrValGlyProThrGlnAsnArgIleLeuArgLeuSerLysGluLeuGlyLeuGluThrTyrLys      90

GTGAACGTAAATGAGCGTCTTGTACAGTATGTCAAGGGGAAAACTTACCCATTCCGAGGAGCCTTTCCTCCGGTATGGAATCCTATCGCA     386
ValAsnValAsnGluArgLeuValGlnTyrValLysGlyLysThrTyrProPheArgGlyAlaPheProProValTrpAsnProIleAla     120

TATTTGGATTACAACAACCTGTGGCGGACAATGGATAACATGGGGAAGGAGATTCCAGCAGATGCACCATGGGAGGCCCCACATGCCGAG     476
TyrLeuAspTyrAsnAsnLeuTrpArgThrMetAspAsnMetGlyLysGluIleProAlaAspAlaProTrpGluAlaProHisAlaGlu     150

GAATGGGACAAGATGACTATGAAGGATCTTATCGATAAAATCTGCTGGACAAAGACTGCTAGGAGGTTTGCATCCCTCTTTGTGAATATC     566
GluTrpAspLysMetThrMetLysAspLeuIleAspLysIleCysTrpThrLysThrAlaArgArgPheAlaSerLeuPheValAsnIle     180

AATGTGACCTCTGAGCCCCACGAGGTGTCTGCGCTCTGGTTCCTGTGGTATGTGAAGCAGTGCGGAGGTACCACTCGGATATTCTCGGTG     656
AsnValThrSerGluProHisGluValSerAlaLeuTrpPheLeuTrpTyrValLysGlnCysGlyGlyThrThrArgIlePheSerVal     210

ACCAATGGAGGCCAGGAACGGAAGTTTGTGGGAGGATCCGGTCAAGTGAGTGAACGGATAATGGAGCGCCTTGGGGACAGAGTCAAGCTG     746
ThrAsnGlyGlyGlnGluArgLysPheValGlyGlySerGlyGlnValSerGluArgIleMetGluArgLeuGlyAspArgValLysLeu     240

AAGCGCCCTGTCACCTATGTTGACCAGTCAGATGACAACATCATCATAGAGACACTGAATCATGAACTTTATGAGTGCAAATACGTGATT     836
LysArgProValThrTyrValAspGlnSerAspAspAsnIleIleIleGluThrLeuAsnHisGluLeuTyrGluCysLysTyrValIle     270

AGTGCCATCCCTCCAACTTTGACTGCCAAGATCCACTTCAGACCAGAGCTTCCATCAGAGAGAAATCAGTTAATTCAGCGTCTTCCAATG     926
SerAlaIleProProThrLeuThrAlaLysIleHisPheArgProGluLeuProSerGluArgAsnGlnLeuIleGlnArgLeuProMet     300

GGAGCTATCATCAAGTGTATGATGTATTACAAGGAGGCCTTTTGGAAGAAGAAGGATTACTGTGGCTGTATGATCATTGAAGACGAGGAA    1016
GlyAlaIleIleLysCysMetMetTyrTyrLysGluAlaPheTrpLysLysLysAspTyrCysGlyCysMetIleIleGluAspGluGlu     330

GCTCCAATTTCAATAACCCTGGATGACACCAAGCCAGATGGATCACTGCCCGCTATCATGGGCTTCATACTTGCCAGAAAAGCTGACCGA    1106
AlaProIleSerIleThrLeuAspAspThrLysProAspGlySerLeuProAlaIleMetGlyPheIleLeuAlaArgLysAlaAspArg     360

CTTGCCAAGCTCCATAAAGAAATAAGGAAGAGGAAAATCTGTGAGCTCTATGCCAAAGTGCTAGGATCCCAAGAAGCTTTACAACCCGTA    1196
LeuAlaLysLeuHisLysGluIleArgLysArgLysIleCysGluLeuTyrAlaLysValLeuGlySerGlnGluAlaLeuGlnProVal     390

CACTATGAAGAGAAGAACTGGTGCGAGGAGCAGTACTCCGGGGGGCTGCTATACCGCCTACTTCCCCCCTGGGATCATGACTCACTACGGA    1286
HisTyrGluGluLysAsnTrpCysGluGluGlnTyrSerGlyGlyCysTyrThrAlaTyrPheProProGlyIleMetThrHisTyrGly     420

AGGGTGATCCGCCAGCCATTTGGCAGGATTTACTTTGCTGGCACTGAGACGGCAACACACTGGAGCGGTTATATGGAAGGAGCTGTAGAG    1376
ArgValIleArgGlnProPheGlyArgIleTyrPheAlaGlyThrGluThrAlaThrHisTrpSerGlyTyrMetGluGlyAlaValGlu     450

GCTGGGGAACGGACAGCTAGAGAGGTCTTAAATGCTCTCGGGAGGGTGGCAGAGAAAGACCTGAAGACCCAGGAACCTGAATCAAAGGAT    1466
AlaGlyGluArgThrAlaArgGluValLeuAsnAlaLeuGlyArgValAlaGluLysAspLeuLysThrGlnGluProGluSerLysAsp     480

GTTCCAGCTATGGAAATCACCCACACCTTCTGGGAGAGGAACCTGCCATCGGTGACAGGCCTGCTGAAGCTCATTGGATTTACCACATCA    1556
ValProAlaMetGluIleThrHisThrPheTrpGluArgAsnLeuProSerValThrGlyLeuLeuLysLeuIleGlyPheThrThrSer     510

GTAACTGCCCTGTGGATTGTGGCGTACAAATTCAGGCTGCTGAGACGATCCTGAGTCTTCAGCCCCACACTCTCTGCTTACTCTTCCCCA    1646
ValThrAlaLeuTrpIleValAlaTyrLysPheArgLeuLeuArgArgSer***

GCACCATCAAAAGCAAAATGTCGACAAAGGCTGTGTCATTGGACCATCTTTAAATGCACTGATTTAAGTTTAGTCTCAGTCAGTGTCATA    1736
AGTAAAATCAACCCAAAGAATCACCTCATTAATTTCAATAAGATCAAGCTCCATCATGTTATTTGCCTGGGTAGACTAACTTGTATTGGT    1826
TGGTAGAATAAAACCTGTGATTAAGTCCTTGATTTCAAGATGTCTAATTGAAGTGCTGATCAGAAACCAAAAAAAAAAAAAAAAAAAAA    1914
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Fig. 1. Nucleotide and deduced amino acid sequences of canine MAOA. The deduced amino acid sequence is shown under the nucleotide sequence. Nucleotides are numbered above and amino acids are numbered below from the first methionine. The cDNA for canine MAOA contains 1584 nucleotides encoding 527 amino acids. The boxed amino acids indicate the FAD-binding site. A putative polyadenylation signal (AATAAA) is underlined.

the amount of mRNA was equal in each sample blotted (Fig. 3).

DISCUSSION

For both MAOA and MAOB cDNA, the canine nucleotide sequences were very similar to those of the other species thus far investigated [6, 8, 12, 15], indicating that MAO

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                                                                                1 TGGCCTACTGGAGACGGCCGAGCGGCGGGCACCGGGGCTGCCCGCAGCCAGGCGAGCGCC      60

ATGAGCGGCAAGTGCGACGTGGTCGTGGTGGGGGGCGGCATCTCAGGCATGGCAGCAGCCAAACTTCTGCATGATTTTGGCCTGAACGTG     150
MetSerGlyLysCysAspValValValValGlyGlyGlyIleSerGlyMetAlaAlaAlaLysLeuLeuHisAspPheGlyLeuAsnVal      30

GTTGTTCTGGAGGCCCGGGACCGAGTGGGAGGCAGGACTTACACCATCAGGAACCAAAAGGTTAAATATTTGGACCTTGGAGGATCTTAT     240
ValValLeuGluAlaArgAspArgValGlyGlyArgThrTyrThrIleArgAsnGlnLysValLysTyrLeuAspLeuGlyGlySerTyr      60

GTTGGGCCAACTCAGAATTGTATCTTAAGGTTAGCCAAGGAGCTAGGATTGGAGACCTACAAAGTGAATGAAGTAGAGCGTCTCATCCAT     330
ValGlyProThrGlnAsnCysIleLeuArgLeuAlaLysGluLeuGlyLeuGluThrTyrLysValAsnGluValGluArgLeuIleHis      90

CATGTAAAGGGCAAATCGTACCCCTTCAGGGGCCCCTTCCCACCTGTATGGAACCCAATTGCATACCTAGATCATAACAACCTCTGGAGG     420
HisValLysGlyLysSerTyrProPheArgGlyProPheProProValTrpAsnProIleAlaTyrLeuAspHisAsnAsnLeuTrpArg     120

ACGATGGACGACATGGGGCGAGAGATTCCCAGTGATGCCCCATGGAAGGCACCTCTTGCGGAGGAGTGGGACCACATGACAATGAAGGAG     510
ThrMetAspAspMetGlyArgGluIleProSerAspAlaProTrpLysAlaProLeuAlaGluGluTrpAspHisMetThrMetLysGlu     150

CTTCTGGACAAGATCTGCTGGACAGAATCTGCCAAGCAACTTGCTACTCTCTTTGTGAATCTGTGTGTCACTGCAGAGACCCATGAGGTC     600
LeuLeuAspLysIleCysTrpThrGluSerAlaLysGlnLeuAlaThrLeuPheValAsnLeuCysValThrAlaGluThrHisGluVal     180

TCTGCTCTCTGGTTCCTGTGGTATGTGAAGCAGTGTGGAGGCACGACCAGGATCATCTCAACAACCAATGGAGGGCAGGAGAGAAAATTT     690
SerAlaLeuTrpPheLeuTrpTyrValLysGlnCysGlyGlyThrThrArgIleIleSerThrThrAsnGlyGlyGlnGluArgLysPhe     210

GTGGGCGGATCTGGTCAAGTGAGTGAACGGATAATGGACCTCCTTGGAGACCAAGTGAAGCTGGAGAGGCCTGTGACCCACATTGACCAG     780
ValGlyGlySerGlyGlnValSerGluArgIleMetAspLeuLeuGlyAspGlnValLysLeuGluArgProValThrHisIleAspGln     240

ACAGGAGAAAATGTCCTTGTGGAGACCCTAAACCATGAGGTGTATGAGGCTAAGTATGTGATTAGTGCCATTCCTCCTACTCTGGGCATG     870
ThrGlyGluAsnValLeuValGluThrLeuAsnHisGluValTyrGluAlaLysTyrValIleSerAlaIleProProThrLeuGlyMet     270

AAGATTCATTTCAACCCCCCTCTGCCAATGATGAGAAACCAGCTGATCACTCGTGTGCCTTTGGGCTCAGTCATCAAGTGCATAGTTTAT     960
LysIleHisPheAsnProProLeuProMetMetArgAsnGlnLeuIleThrArgValProLeuGlySerValIleLysCysIleValTyr     300

TATAAAGAGCCCTTCTGGAGGAAAAAGGATTACTGTGGAACCATGATTATCGAAGGAGAGGAAGCTCCAATTGCTTACACGTTGGATGAT    1050
TyrLysGluProPheTrpArgLysLysAspTyrCysGlyThrMetIleIleGluGlyGluGluAlaProIleAlaTyrThrLeuAspAsp     330

ACCAAACCTGATGGCAACTATGCCGCCATAATGGGATTTATCCTTGCCCACAAAGCCAGAAAACTGGCACGTCTTACCAAAGATGAAAGG    1140
ThrLysProAspGlyAsnTyrAlaAlaIleMetGlyPheIleLeuAlaHisLysAlaArgLysLeuAlaArgLeuThrLysAspGluArg     360

ATGAAGAAACTTTGTGAGCTCTATGCAAAAGTCCTGGGTTCCCAAGAAGCTTTGCAGCCCGTACACTACGAAGAGAAGAACTGGTGCGAG    1230
MetLysLysLeuCysGluLeuTyrAlaLysValLeuGlySerGlnGluAlaLeuGlnProValHisTyrGluGluLysAsnTrpCysGlu     390

GAGCAGTACTCCGGGGGCTGCTACACCACCTACTTTCCCCCTGGGATCATGACTCAATATGGAAGGGTTCTACGACAGCCTGTGGGCAGG    1320
GluGlnTyrSerGlyGlyCysTyrThrThrTyrPheProProGlyIleMetThrGlnTyrGlyArgValLeuArgGlnProValGlyArg     420

ATTTATTTTGCAGGCACGGAGACTGCCACCCACTGGAGTGGCTACATGGAGGGCGCTGTGGAGGCTGGAGAGAGAGCGGCCCGAGAGATC    1410
IleTyrPheAlaGlyThrGluThrAlaThrHisTrpSerGlyTyrMetGluGlyAlaValGluAlaGlyGluArgAlaAlaArgGluIle     450

CTGCATGCCATGGGGAAGATCCCAGAGGATGAAATCTGGCAGTCAGAACCAGAATCAGTGGATGTCCCTGCGCAGCCCATCACCACGACC    1500
LeuHisAlaMetGlyLysIleProGluAspGluIleTrpGlnSerGluProGluSerValAspValProAlaGlnProIleThrThrThr     480

TTCTTGGAGAGACATTTGCCCTCTGTGCCAGGCCTGCTGAGGCTGATCGGATTGACCGCCATCTTTTCTGCAACTGCTCTTGGCGTCCTG    1590
PheLeuGluArgHisLeuProSerValProGlyLeuLeuArgLeuIleGlyLeuThrAlaIlePheSerAlaThrAlaLeuGlyValLeu     510

GCACACAAAAGGGGTCTGCTTGTGCGGGTCTAA                                                               1623
AlaHisLysArgGlyLeuLeuValArgVal***                                                                521

AGAGAAAGCGTCTGTAACTACACCTTCTTCTTACTCTATCTGGTGTGTGGGTTTGGGGAAGGAATTGCAGTAAGGTTCCAAGAGGACATC    1713
GCGACTGTAGAATGAGGTGAGGAGCATGAAGATAAGTCAGACTTTCCACCACAGGAAACACAGTATGTCCTTTGCTGCTTGGCCCCTGCA    1803
TATTGTCCCTTACCTAGCTTTGCGCTCTGTCTTGCCCATTTCCCAGAATCTTTACCATAGTTAAGCAAGCTCTCACTGCCCACGACGCAC    1893
CTTGCCCACGCACAAAAAAAACTAGTTTTTTCCCCCACCTTTGTGGCTCTGTGCTTGTCCTTTCTCCTACATGTAACATCCTCTTTGTCCA    1983
AGTTCTCCCCACTTGTCCTTAGAATCCTGTATTGTGATAGCTGGGCCTTGAAGAACATCTAGTCTTCACCTTCTCTTGTAGTGTTGATCA    2073
AGCCAAGGCCAGGAGAGGTGGAACTTAATTGCCCAAGGTCCACAATAAGACACTGATATTTGGAGGACTAGAACACAGGTCACAGGTCCA    2163
TTGCCTCTTCCGCCCCACAGGATGTATGCCTCAACCACCTTCTTCCACTCCCTGCCATGGTCACCGATGGTGTCCCTTTGTGTGGGTTTA    2253
CTCTGCACTAGGTTGTTATGTGCTACTCAGTGCTACTCAGTATATATCTGTAAGTCTTATTGTCCTGTGAAATGTCAGCTGAAGCTGATT    2343
TTAATTTCTTTCAGGATAGCAATCTTGTCTTTTCTTCCTTTTTGTATCCTCCCATTATGTCTGGATACAAAGGTCATTCATTACACACTAG    2433
CAAGTAAAAATAAAGTGGATTGACCAGAAAAAAAAAA                                                           2470
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Fig. 2. Nucleotide and deduced amino acid sequences of MAOB. The deduced amino acid sequence is shown under the nucleotide sequence. Nucleotides are numbered above and amino acids are numbered below from the first methionine. The cDNA for canine MAOB contains 1563 nucleotides encoding 520 amino acids. The boxed amino acids indicate the FAD-binding site. A putative polyadenylation signal (AATAAA) is underlined.

Table 1. Nucleotide and amino acid sequence similarity of canine MAOA and MAOB to other mammalian species

	Nucleotide	Amino acid
MAOA		
bovine	86.7%	89.6%
human	86.8%	89.8%
rat	81.6%	85.6%
MAOB		
human	91.8%	95.0%
rat	85.3%	88.5%

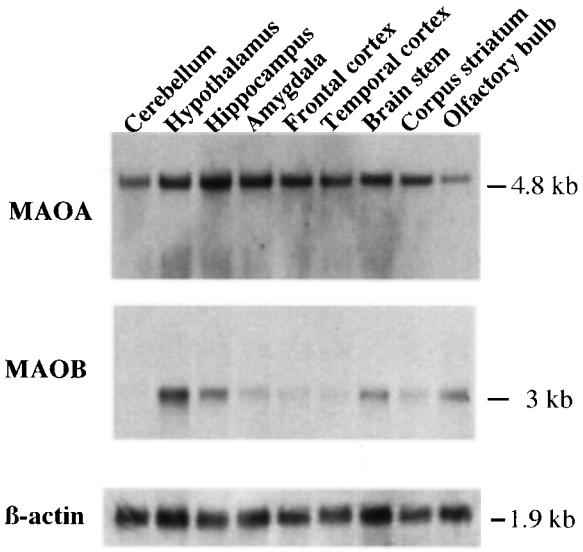


Fig. 3. Northern blot analysis of canine monoamine oxidase mRNA in various brain regions. From each region, three micrograms of total RNA was loaded on 1% agarose MOPS-formaldehyde gel for electrophoresis and transferred to nylon membranes. The RNA was then hybridized with DIG-11-UTP-labeled canine MAOA, MAOB and β-actin cRNA probes.

is a pivotal enzyme well conserved throughout mammalian evolution. The FAD-binding site, to which the obligatory cofactor FAD is covalently bound, may be crucial for enzymatic activity of MAO, since the site was completely conserved in four species beyond the MAO subtypes.

The calculated molecular masses of deduced proteins of canine MAO isoforms differed between subtypes; MAOA and MAOB were 60.0 and 58.4 kDa, respectively. This implies that these two enzymes are the products of different genes. The comparison of amino acid sequences between canine MAOA and MAOB, however, showed a relatively high similarity (> 70%) as has been reported for human MAO genes. This suggests that these two genes may have a common ancestral gene. Through evolutionary changes, monoamines diversified to meet different important needs, two different enzymes might have been created to catabolize biogenic amines more successfully on the basis of amine type and tissue-specific distribution.

The Northern blot analysis of canine MAOA mRNA revealed among species difference in its expression patterns. Messages for canine MAOA mRNA were strongly detected in the hippocampus, the frontal cortex and the cerebellum. In humans, on the other hand, MAOA mRNA was barely detected in the hippocampus and cerebellum [18]. Likewise, rat MAOA mRNA transcripts were barely detected in the cerebrum [12]. These results suggest that MAO A mRNA has a species-specific pattern of gene expression in the brain.

The distribution pattern of canine MAOB mRNA in the brain was quite different from that of canine MAOA. Large amounts of canine MAOB mRNA were detected in the hippocampus and brain stem. However, under the hybridization condition used in the present study, mRNA of MAOB was not detectable in the cerebellum. In a human study, MAOB transcripts were detected clearly in the cerebellum but not in the hippocampus [18]. These results suggest that MAOB mRNA also has a species-specific manner of gene expression.

Regarding the subtype-specific distribution pattern of MAO gene expression in the canine brain, MAOA mRNA was found broadly in various regions of the brain, whereas MAOB mRNA expression was limited to specific areas of the brain in which catecholaminergic and serotonergic neurons were located. Boulton and Eisenhoger [3] discovered that MAOA was present in the neural cell bodies of all the catecholaminergic neurons, while MAOB in serotonergic neurons, and in non-neural cells, such as astrocytes in the human brain [3]. These results led us to hypothesize that MAOB could be detected in the astrocytes-rich regions such as cortex and cerebellum, but canine MAOB was not detected in these areas in the present study. This may suggest that MAOA is playing some complementary roles for the function of MAOB in the canine brain. As a supporting evidence for this, 1-methyl-4-aryl-1,2,3,6-tetrahydropyridinyl (a substrate for MAOB) was shown to be catalyzed by MAOB in the brain of in non-human primates, whereas it was catalyzed mainly by MAOB and partially by MAOA in rats and dogs [7]. It remains to be elucidated which type of MAO isoform contributes to catalyze substrates and in what kind of cell types MAOA and/or MAOB are expressed in the canine brain.

Cognitive deficits have recently been demonstrated in monkeys with dopamine depletion in the prefrontal cortex [1]. In another study, therapy with l-deprenyl (an irreversible MAOB inhibitor) improved the performance of aged dogs that had exhibited cognitive dysfunction [13]. Treatment with l-deprenyl for cognitive dysfunction is thought to operate by inhibiting dopamine deamination by MAOB in the brain [13]. The present study revealed that canine MAOB mRNA was localized specifically in the hypothalamus, the hippocampus and the brain stem, including the raphe nuclei and the locus ceruleus. Dopamine depletion in these brain regions may be related to cognitive deficits in

aged dogs, as was the case with dopamine depletion in the prefrontal cortex in monkeys [1]. Cell-type-specific distribution patterns of MAO in the canine brain remain to be studied. For instance, in situ hybridization with MAO mRNAs in normal dogs and in dogs with cognitive dysfunction would provide further insights into the role of classical monoamines in aged dogs with normal cognitive function as well as in those with cognitive dysfunction.

The abnormal aggression reported in humans with complete MAOA deficiency was attributed to a point mutation in the gene encoding MAOA [4], but it is unclear whether this kind of genetic background for aggressive behavior is also the case for dogs. The present study revealed that the expression pattern of canine MAOA was different from that of human MAOA. Because the regulation of MAOA gene expression and its behavioral effects are largely unexplored, further study is needed to develop our understanding of canine MAO function and its postulated roles in the progress of canine cognitive dysfunction and abnormal aggression.

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REFERENCES

1. Arnsten, A. F. 1993. Catecholamine mechanisms in age-related cognitive decline. *Neurobiol. Aging* **14**: 639–641.
2. Bach, A. W., Lan, N. C., Johnson, D. L., Abell, C. W., Bembenek, M. E., Kwan, S. W., Seeburg, P. H. and Shih, J. C. 1988. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc. Natl. Acad. Sci. U. S. A.* **85**: 4934–4938.
3 Boulton, A. A. and Eisenhofer, G. 1998. Catecholamine metabolism. From molecular understanding to clinical diagnosis and treatment. Overview. *Adv. Pharmacol.* **42**: 273–292.
4 Brunner, H. G., Nelen, M., Breakefield, X. O., Ropers, H. H. and van Oost, B. A. 1993. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science* **262**: 578–580.
5 Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J. C. *et al.* 1995. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science.* **268**: 1763–1766.
6 Hsu, Y. P., Weyler, W., Chen, S., Sims, K. B., Rinehart, W. B., Utterback, M. C., Powell, J. F. and Breakefield, X. O. 1988. Structural features of human monoamine oxidase A elucidated from cDNA and peptide sequences. *J. Neurochem.* **51**: 1321–1324.
7 Inoue, H., Castagnoli, K., Van Der Schyf, C., Mabic, S., Igarashi, K. and Castagnoli, N. Jr. 1999. Species-dependent differences in monoamine oxidase A and B-catalyzed oxidation of various C4 substituted 1-methyl-4-phenyl-1,2,3, 6- tetrahydropyridinyl derivatives. *J. Pharmacol. Exp. Ther.* **291**: 856–864.
8 Ito, A., Kuwahara, T., Inadome, S. and Sagara, Y. 1988. Molecular cloning of a cDNA for rat liver monoamine oxidase B. *Biochem. Biophys. Res. Commun.* **157**: 970–976.
9 Johnston, J. P. 1968. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **17**: 1285–1297.
10 Klinteberg, B., Levander, S. E., Oreland, L., Asberg, M. and Schalling, D. 1987. Neuropsychological correlates of platelet monoamine oxidase (MAO) activity in female and male subjects. *Biol. Psychol.* **24**: 237–252.
11 Knoll, J. and Magyar, K. 1972. Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmacol.* **5**: 393–408.
12 Kuwahara, T., Takamoto, S. and Ito, A. 1990. Primary structure of rat monoamine oxidase A deduced from cDNA and its expression in rat tissues. *Agric. Biol. Chem.* **54**: 253–257.
13 Milgram, N. W., Ivy, G. O., Head, E., Murphy, M. P., Wu, P. H., Ruehl, W. W., Yu, P. H., Durden, D. A., Davis, B. A., Paterson, I. A. *et al.* 1993. The effect of L-deprenyl on behavior, cognitive function, and biogenic amines in the dog. *Neurochem. Res.* **18**: 1211–1219.
14 Minamiura, N. and Yasunobu, K. T. 1978. Bovine liver monoamine oxidase. A modified purification procedure and preliminary evidence for two subunits and one FAD. *Arch. Biochem. Biophy.* **189**: 481–489.
15 Powell, J. F., Hsu, Y. P., Weyler, W., Chen, S. A., Salach, J., Andrikopoulos, K., Mallet, J. and Breakefield, X. O. 1989. The primary structure of bovine monoamine oxidase type A. Comparison with peptide sequences of bovine monoamine oxidase type B and other flavoenzymes. *Biochem. J.* **259**: 407–413.
16 Ruehl, W. W., Bruyette, D. S., DePaoli, A., Cotman, C. W., Head, E., Milgram, N. W. and Cummings, B. J. 1995. Canine cognitive dysfunction as a model for human age-related cognitive decline, dementia and Alzheimer's disease: clinical presentation, cognitive testing, pathology and response to 1-deprenyl therapy. *Prog. Brain Res.* **106**: 217–225.
17 Shih, J. C. 1991. Molecular basis of human MAO A and B. *Neuropsychopharmacology* **4**: 1–7.
18 Shih, J. C., Grimsby, J. and Chen, K. 1990. The expression of human MAO-A and B genes. *J. Neural Transm.* (*Suppl.*) **32**: 41–47.