Available online at www.sciencedirect.com
SCIENCE DIRECT®
Developmental Brain Research 148 (2004) 223–232
DEVELOPMENTAL BRAIN RESEARCH
www.elsevier.com/locate/devbrainres

Gene Expression Pattern

Expression of DDAH1 in chick and rat embryos

Takuya Mishima[a], Tsuyoshi Hamada[b], Kumiko Ui-Tei[c], Fumitaka Takahashi[c], Yuhei Miyata[d], Junko Imaki[a,*], Hidenori Suzuki[d], Kazuo Yamashita[a]

[a] *Department of Anatomy, Nippon Medical School, Bunkyo, Tokyo 113-8602, Japan*
[b] *Creative Research Promoting Program Stem Cell Translational Laboratory, Riken Kobe Institute Center for Developmental Biology, Chuo, Kobe 650-0047, Japan*
[c] *UPBSB, Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Bunkyo, Tokyo 113-0033, Japan*
[d] *Department of Pharmacology, Nippon Medical School, Bunkyo, Tokyo 113-8602, Japan*

Accepted 10 September 2003

Abstract

Dimethylarginine dimethylaminohydrolase 1 (DDAH1) is an enzyme that metabolizes methylated arginine to citrulline and methylamine, thus working to produce nitric oxide (NO). We isolated a gene encoding chick DDAH1. In situ hybridization analysis revealed characteristic DDAH1 mRNA expression in the embryonic spinal cord, which was especially strong in the ventral horn and dorsal root ganglion (DRG). DDAH1 was also detected in the brain, kidney, digestive tract, and in other tissues. We examined the expression pattern of DDAH1 in developing rats and compared this with the expression pattern in chicks. The expression pattern in the rats was very similar to that in the chicks, but there were some differences between the chicks and rats in the amount of DDAH1 detected in the heart, liver, lung, and DRG. We also investigated neural nitric oxide synthase (nNOS) mRNA expression patterns in rat embryos. The DDAH1 expression patterns were completely different from nNOS expression patterns. Our study suggests that DDAH1 plays an important role in development.

Keywords: Central nervous system; Chick; DDAH; Development; Dorsal root ganglion; Esophagus; Heart; In situ hybridization; Intestine; Kidney; Liver; Lung; Rat; Rectum; Retina; Spinal cord; Stomach

1. Results

1.1. Cloning of chick DDAH1

To identify proteins involved in spinal cord differentiation, we screened for genes, whose expression in chick spinal cords changed between embryonic days 4 (E4) and 10 (E10) using the differential display method, and obtained several cDNA fragments. We selected one of the fragments that showed increasing expression between E4 and E10. Using this fragment as a probe, we isolated full-length cDNA of the gene from the E5 chick cDNA library and sequenced it. This sequence encodes a putative protein composed of 287 amino acids and is homologous to human DDAH1 (85%) and rat DDAH1 (84%) (Fig. 1).

1.2. Expression of DDAH1 mRNA in CNS

1.2.1. Expression in the spinal cords

We examined chicks on days E4, E6, E8, E10, E14 and E18 (Figs. 2A–O and 3) and rats on days E14 and E17 (Figs. 2P–U and 4). Signals were detected in the marginal layer of the spinal cord of the E4 chicks (Figs. 2A–C and 3A–C) and signal intension was found to be in the order of cervical, thoracic and lumbar spinal cord. At chick stage E6, signals were also detected in the marginal layer, especially in the ventral portion, and were first detected in the dorsal root ganglion (DRG) and sympathetic ganglion (SG) (Figs. 2D–F and 3D–F). At chick stages E8 (Figs. 2G–I and 3G–I) and E10 (Figs. 2J–L and 3J–L), the expression pattern was almost identical to that of E6. At E14 (Fig. 2M) and E18 (Fig. 2N,O) in the chicks, the signals became gradually

* Corresponding author. Tel.: +81-3-3822-2131; fax: +81-3-5685-3052.
E-mail address: jimaki@nms.ac.jp (J. Imaki).

0165-3806/$ - see front matter © 2004 Elsevier B.V. All Rights Reserved.
doi:10.1016/j.devbrainres.2003.09.021

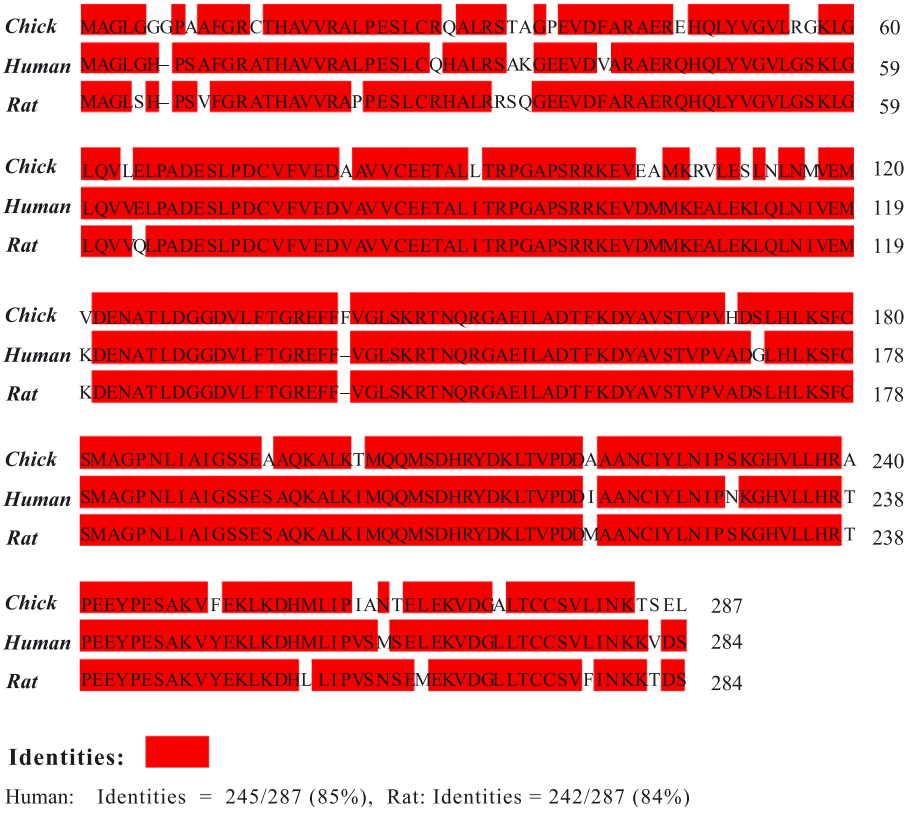

Fig. 1. Comparison of amino acid sequences of DDAH1 genes. Accession numbers for DDAH1: chick, AB095027; human, NM_012137; rat, NM_022297.

weaker in the gray matter, but were still rather strong in DRG and SG. In contrast, in the rats on both E14 (Figs. 2P–R and 4A–F) and E17 (Figs. 2S–U and 4G–J), signals were detected in the gray matter, especially in the ventral portion (probably coincident with motor neurons on E17), and SG, but interestingly, were detected very weakly or not at all in the DRG.

1.2.2. Expression in the rat brain

We examined the rat brains on E14 and E17 (Fig. 5). On E14, signals were detected in the entire ventricular zone (VZ) (Fig. 5A,B,D) and the tegmentum (Tg) (Fig. 5C). On E17, signals were observed in the VZ (Fig. 5E–G,I,J), the choroid plexus (ChP) (Fig. 5G–J), the ciliary ganglion (CG) (Fig. 5F), the inferior olive (IO) (Fig. 5I), the 7th cranial nerve (7th) (Fig. 5H), the interpeduncular nucleus (IpN) (Fig. 5H), the red nucleus (RN) (Fig. 5H), the dorsal tegmental nucleus (DTN) (Fig. 5I) and the nucleus of the lateral lemniscus (NLL) (Fig. 5H).

1.3. Expression of DDAH1 mRNA in thoracic organs

We examined thoracic organs in the chicks on E4, E6, E8 and E10 (Figs. 3B–E,G,H,J,K and 6A–E) and thoracic organs in the rats on E14 and E17 (Figs. 4A,B,G,H and 6F–J). At chick stage E4, strong signals were observed in the aorta (Fig. 6A) and in the esophagus (Fig. 6B). Signals in the aorta in the following stages gradually weakened (Fig. 6C–E). Signals in the esophagus were observed mainly in the smooth muscle and in the mucosa on E4 (Fig. 6B) and E6 (Fig. 6C). In the smooth muscle, signals gradually weakened in the following stages (Fig. 6D,E), while in the mucosa, signals continued to be intense (Fig. 6D,E).

In the rats on E14 and E17, signals were also detected in the aorta (Fig. 6F–H) and in the esophagus (Fig. 6F–H). In the rat esophagus, signals in the mucosa were not as strong as those in the chick mucosa, but the signal patterns were very similar. In the rat respiratory system (Fig. 6F–H) and heart ventricles (Fig. 6I,J), in contrast with the chick respiratory system and heart ventricles, intense signals were detected.

1.4. Expression of DDAH1 mRNA in abdominal organs

We examined abdominal organs in the chicks on E6, E8 and E10 (Figs. 3E,F,H,I,L and 7A–H) and abdominal organs in the rats on E14 and E17 (Figs. 4B–F,I,J and 7I–P). In the chicks, strong signals were detected in the tubules of the kidney (Fig. 7A–C), in the serosa of the stomach (Fig. 7D–F), in the serosa of and in the boundary between the smooth muscle and submucosa of the intestine (Fig. 7G,H), and in the rectum expression patterns were similar to those for signals detected in the intestine (Fig. 7B,C). Moderate signals were detected in the mucosa and

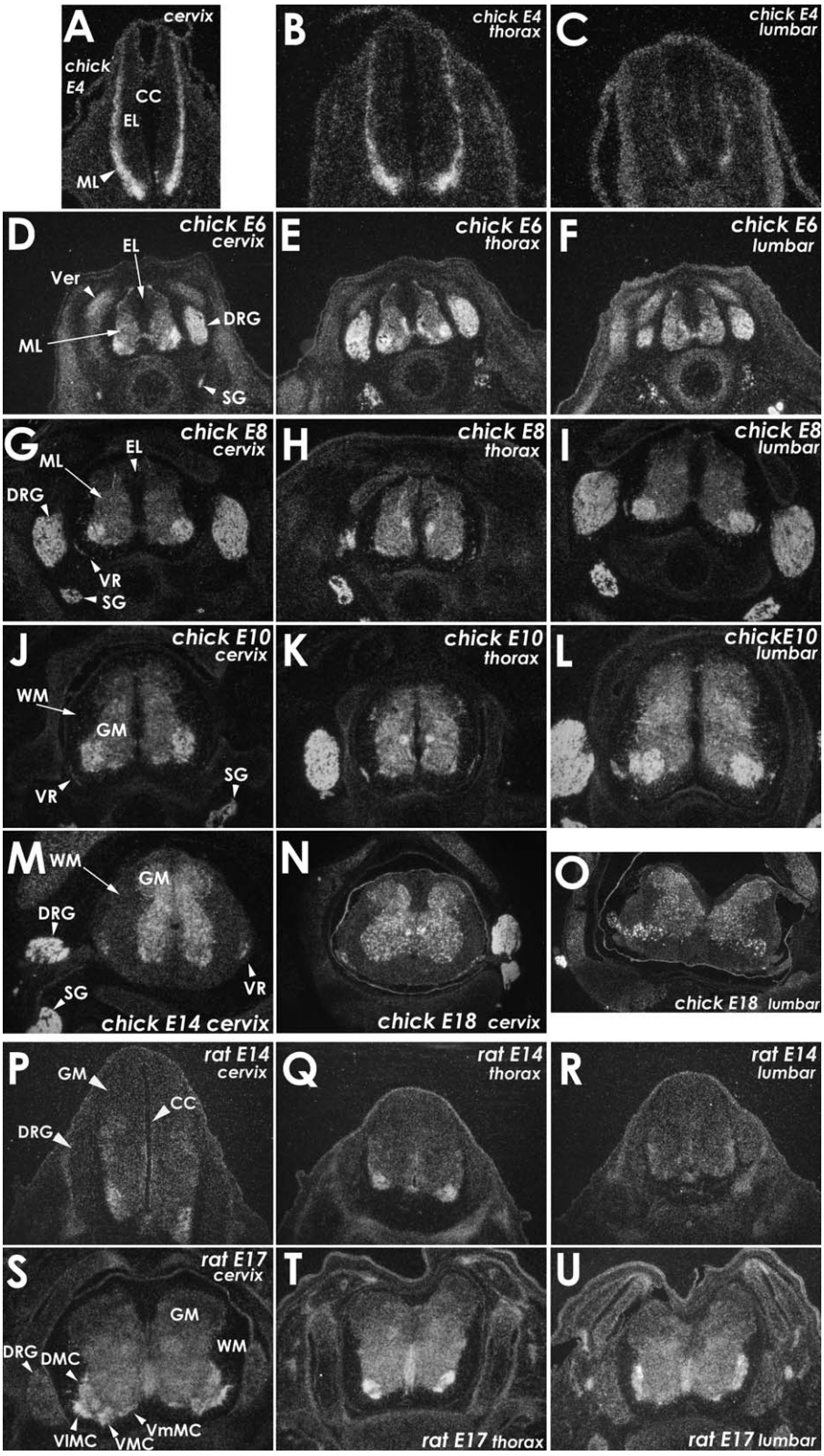

Fig. 2. In situ hybridization analyses of DDAH1 mRNA in the developing spinal cord of the chicks on days E4 (A–C), E6 (D–F), E8 (G–I), E10 (J–L), E14 (M) and E18 (N, O), and of the rats on E14 (P–R) and E17 (S–U). These figures are at the cervical (A, D, G, J, M, N, P, S), thoracic (B, E, H, K, Q, T) or lumbar (C, F, I, L, O, R, U) levels. CC, central canal; DMC, dorsolateral motor columns; DRG, dorsal root ganglion; EL, ependymal layer; GM, gray matter; ML, marginal layer; SG, sympathetic ganglion; Ver, vertebrae; VMC, ventral motor columns; VlMC, ventrolateral motor columns; VmMC, ventromedial motor columns; VR, ventral root; WM, white matter.

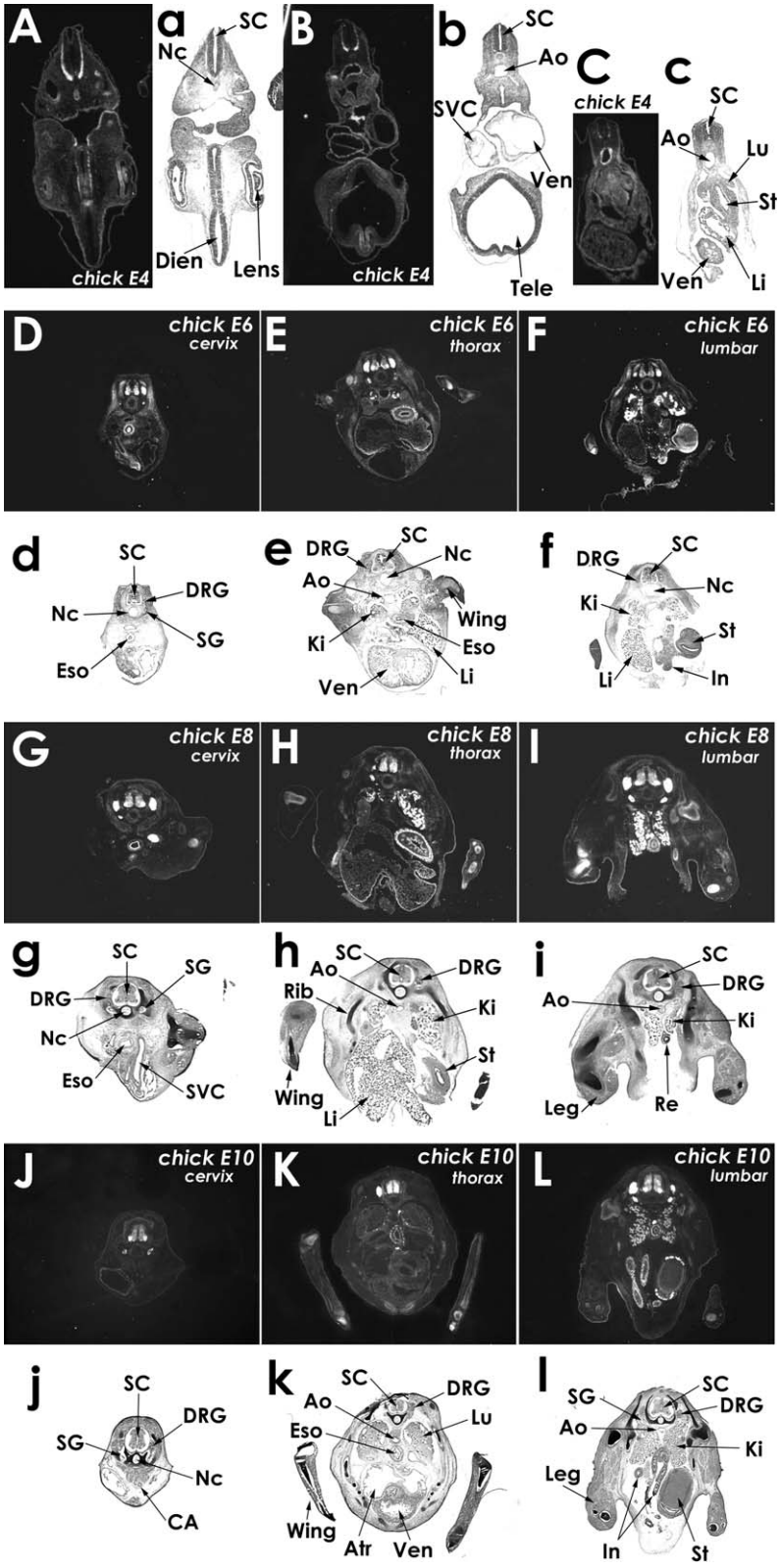

Fig. 3. DDAH1 mRNA expression pattern (A–L) and adjacent Nissl-stained sections (a–l) in the chick bodies on E4 (A–C), E6 (D–F), E8 (G–I) and E10 (J–L). Each section is at the cervical (A, D, G, J), thoracic (B, C, E, H, K) or lumbar (F, I, L) level. Ao, aorta; Atr, atrium; CA, carotid artery; Dien, diencephalon; DRG, dorsal root ganglion; Eso, esophagus; In, intestine; Ki, kidney; Li, liver; Lu, lung; Nc, notochord; Re, rectum; SC, spinal cord; SG, sympathetic ganglion; St, stomach; SVC, superior vena cava; Tele, telencephalon; Ven, ventricle.

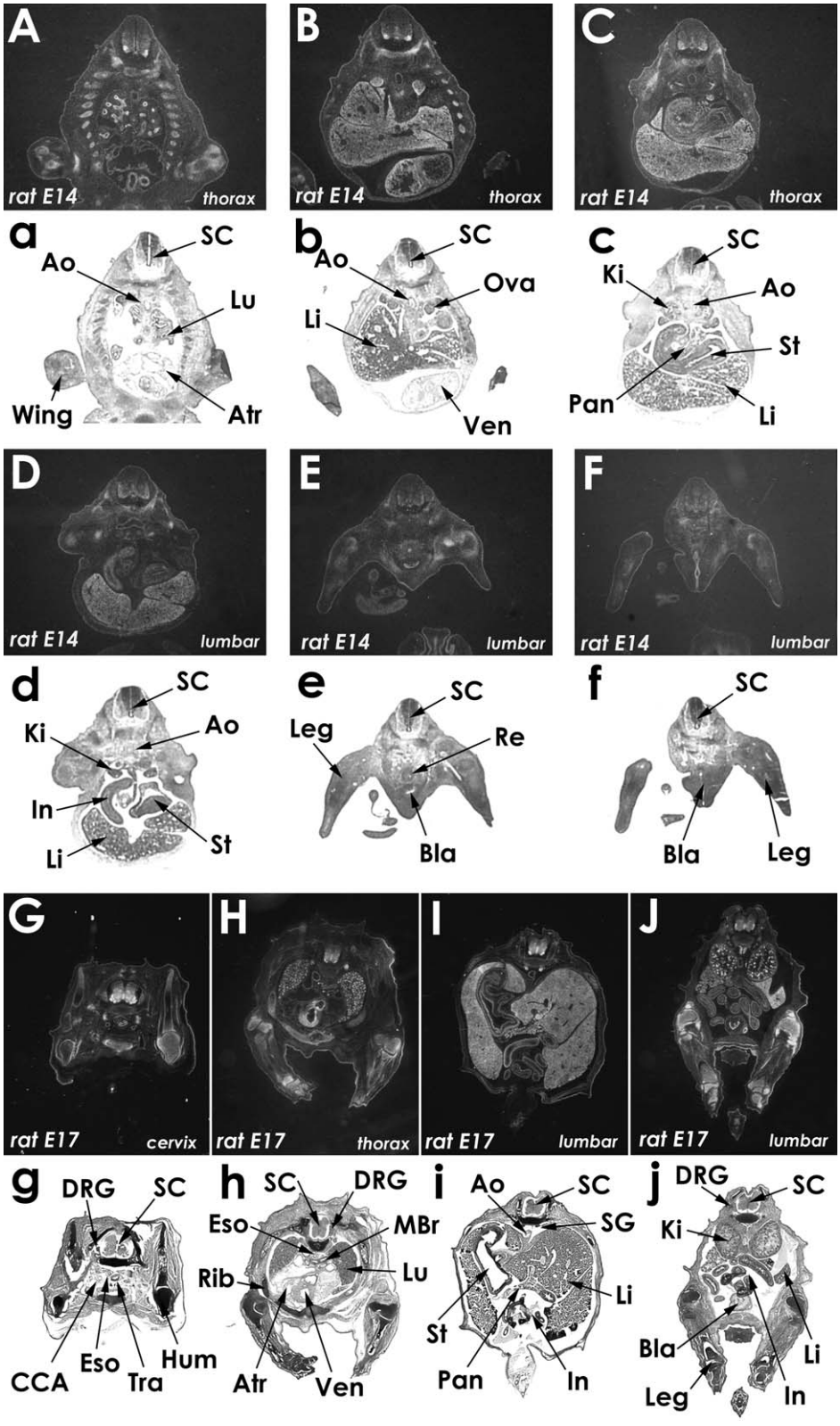

Fig. 4. DDAH1 mRNA expression pattern (A–J) and adjacent Nissl-stained sections (a–j) in the rats bodies on E14 (A–F) and E17 (G–J). Each section is at the cervical (G), thoracic (A–C, H), or lumbar (D–F, I, J) level. Ao, aorta; Atr, atrium; Bla, bladder; CCA, common carotid artery; DRG, dorsal root ganglion; Eso, esophagus; Hum, humerus; In, intestine; Ki, kidney; Li, liver; Lu, lung; MBr, main bronchus; Ova, ovary; Pan, pancreas; Re, rectum; SC, spinal cord; SG, sympathetic ganglion; St, stomach; Tra, trachea; Ven, ventricle.

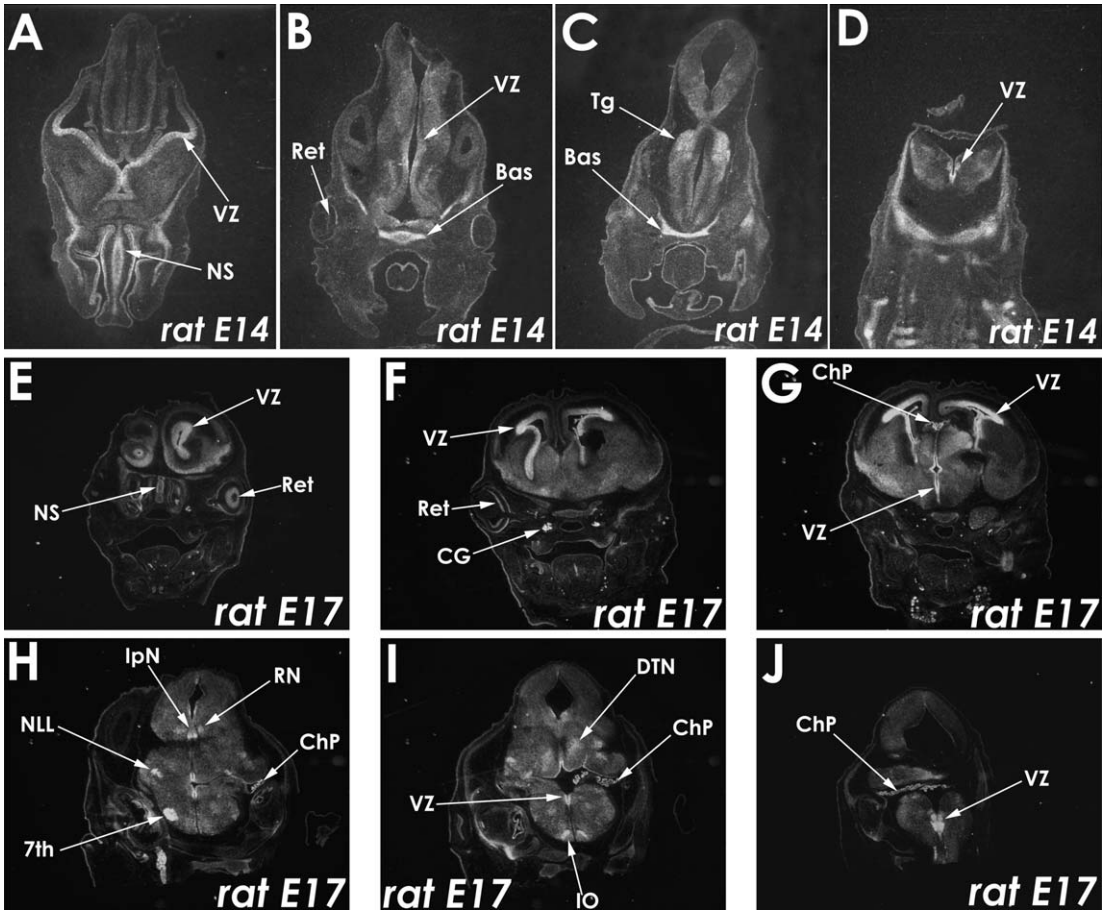

Fig. 5. In situ hybridization analyses of DDAH1 mRNA in rat brain coronal sections on E14 (A–D) and E17 (E–J). 7th, 7th cranial nerve; Bas, basioccipital bone; CG, ciliary ganglion; ChP, choroid plexus; DTN, dorsal tegmental nucleus; IO, inferior olive; IpN, interpeduncular nucleus; NLL, nucleus of the lateral lemniscus; NS, nasal septum; Ret, retina; RN, red nucleus; Tg, tegmentum; VZ, ventricular zone.

smooth muscle of the stomach (Fig. 7D–F), the intestine (Fig. 7G,H) and the rectum (Fig. 7B,C).

In the rats on E14 and E17, a similar expression pattern to that found in the chicks was detected in the kidney (Fig. 7I,J), stomach (Fig. 7K,L), intestine (Fig. 7M) and rectum (Fig. 7N,O), but the signals were not as intense as those found in the chicks. Moreover, in the rats, signals were detected in the liver (Fig. 7P), ovary (Fig. 7I), bladder (Fig. 7M,N), seminal vesicle (Fig. 7O) and pancreas (Fig. 7K,L).

1.5. Expression of DDAH1 mRNA in other organs

Signals were observed in the inner nuclear layer and pigment layer of the retina (Fig. 8A,B), in the salivary gland (Fig. 8C) and in the bones of both chick (Fig. 3I, see i Leg) and rat embryo (Fig. 8D–F).

1.6. Comparison of DDAH1 mRNA and neural nitric oxide synthase (nNOS) mRNA in the brains of rats on E16

DDAH1 mRNA signals were detected mainly in the VZ and DTN (Fig. 9A), and nNOS mRNA signals were detected mainly in the inner nuclear layer and intermediate zone (Fig. 9B). DDAH1 signal expression patterns were completely different from nNOS expression patterns (Fig. 9C). In other organs in rat embryos, DDAH1 expression patterns were different from nNOS expression patterns (data not shown).

2. Materials and methods

Differential display, molecular cloning and sequencing were performed as described previously [2]. At each stage of Wistar rat and White leg-horn chick, embryos was fixed with Zamboni's fixative. Sections of 20 μm thickness were cut on a cryostat and mounted onto silane-coated slides. In situ hybridization was performed with 35S-UTP labeled RNA riboprobes according to the method described by Yoshida et al. [9]. The following primer pairs of DDAH1 were used, forward and backward, respectively: chick DDAH1, AGCATCAGCTGTACGTG and TTTCTCCAGTTCTGTGTTGG, rat DDAH1, AGGCTGATGATGGCTCTGTA and ATCCAGAGTTCGAGACCTTG.

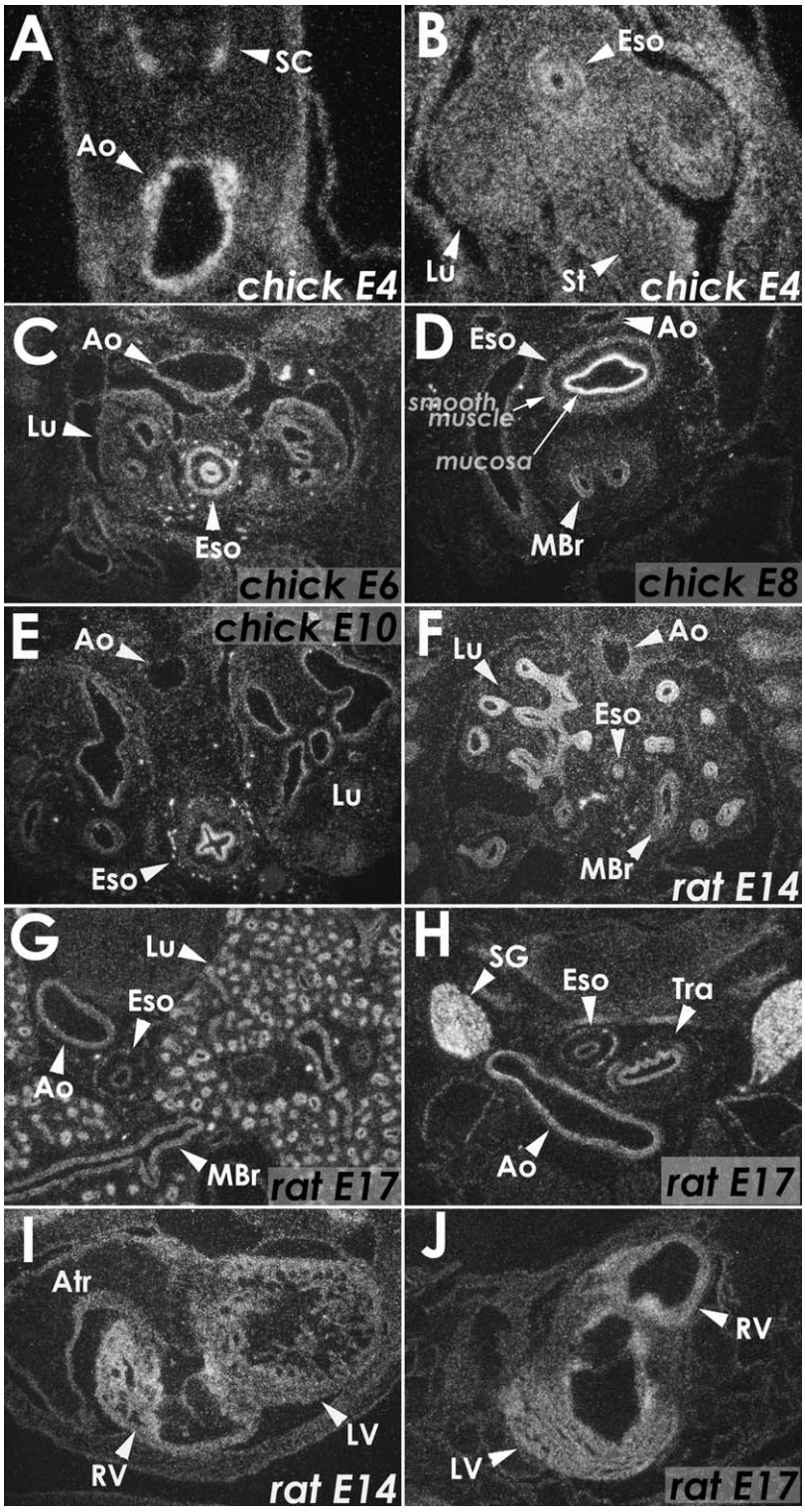

Fig. 6. In situ hybridization analyses of DDAH1 mRNA in the embryonic thoracic organs. A shows the chick aorta on E4. B–G show the mid-thoracic level in the chicks on E4 (B), E6 (C), E8 (D) and E10 (E), and in the rat on E14 (F) and E17 (G), including lung, esophagus, aorta and bronchus. H is an upper thoracic transverse section that includes the aortic arch, esophagus and trachea in the rats on E17. I and J show the rat hearts on E14 and E17, respectively. Ao, aorta; Atr, atrium; Eso, esophagus; LV, left ventricle; Lu, lung; MBr, main bronchus; RV, right ventricle; SC, spinal cord; SG, sympathetic ganglion; St, stomach; Tra, trachea.

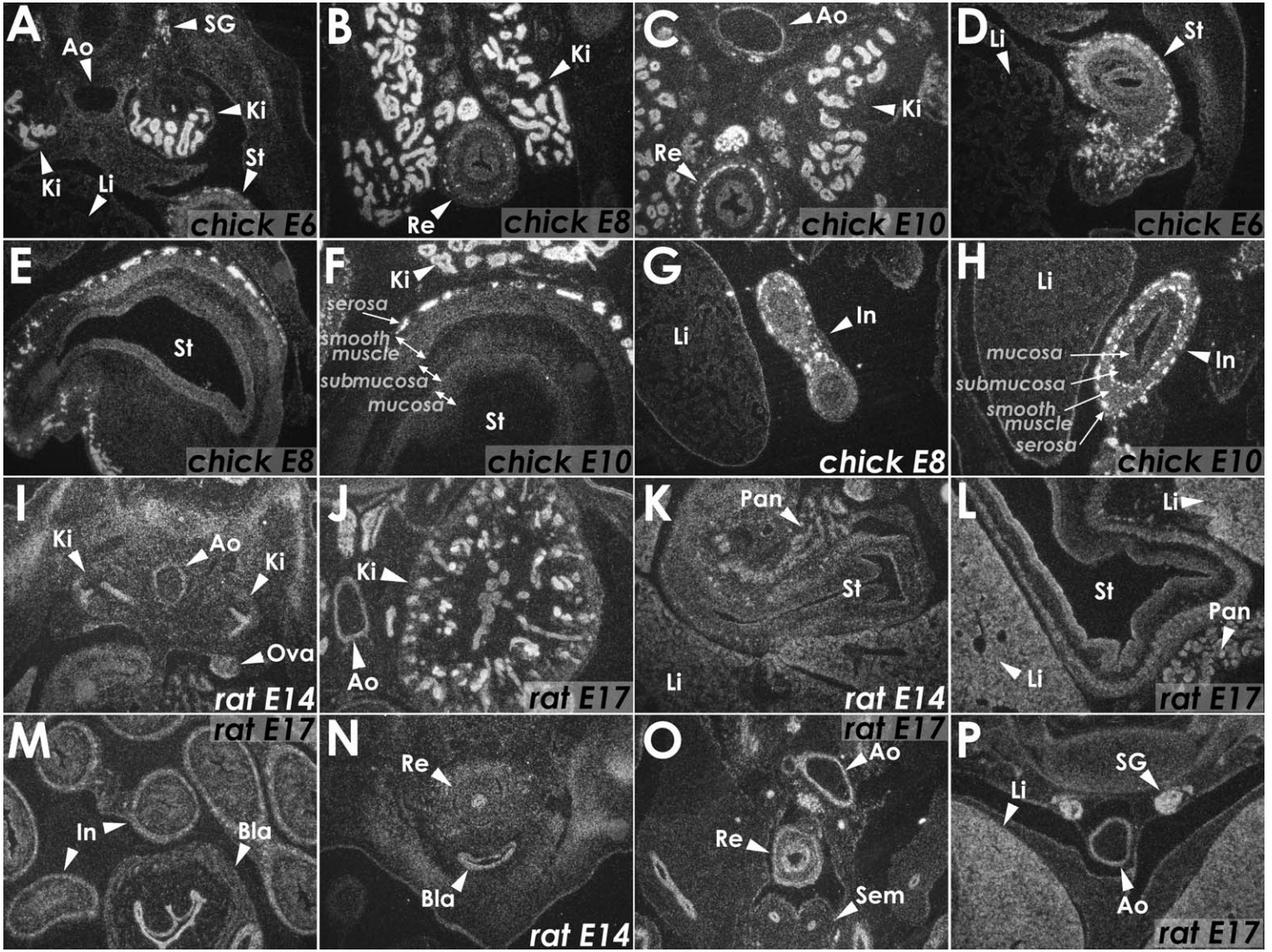

Fig. 7. In situ hybridization analyses of DDAH1 mRNA in the embryonic abdominal organs. A–C, I, J show the kidney and surrounding organs in the chicks on E6 (A), E8 (B) and E10 (C), and in the rats on E14 (I) and E17 (J). D–F, K, L show the stomach and surrounding organs in the chicks on E6 (D), E8 (E) and E10 (F), and in the rats on E14 (K) and E17 (L). G, H, M–O show the enteral organs in the chicks on E8 (G) and E10 (H), and in the rats on E14 (N) and E17 (M, O). P shows the rat abdominal aorta on E17. Ao, aorta; Bla, bladder; In, intestine; Ki, kidney; Li, liver; Ova, ovary; Pan, pancreas; Re, rectum; Sem, seminal vesicle; SG, sympathetic ganglion; St, stomach.

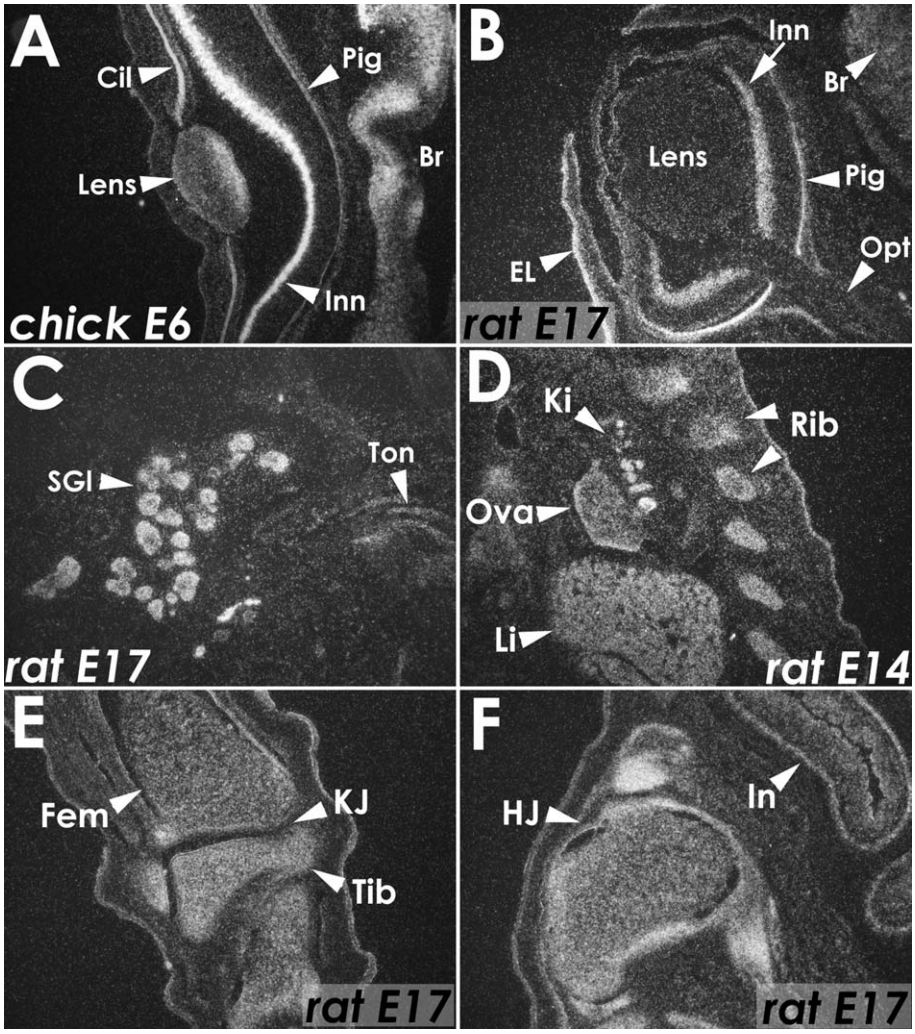

Fig. 8. In situ hybridization analyses of DDAH1 mRNA in other embryonic organs. Signal expression in the eye of the chick on E6 (A) and of the rat on E17 (B); expression in the salivary gland of the rat on E17 (C); expression in the bone of the rat on E14 (D) and E17 (E, F). Br, brain; Cil, ciliary muscle; EL, eyelid; Fem, femur; HJ, hip joint; In, intestine; Inn, inner nuclear layer of retina; Ki, kidney; KJ, knee joint; Li, liver; Opt, optic nerve; Ova, ovary; Pig, pigment layer of retina; SGl, salivary gland; Tib, tibia; Ton, tongue.

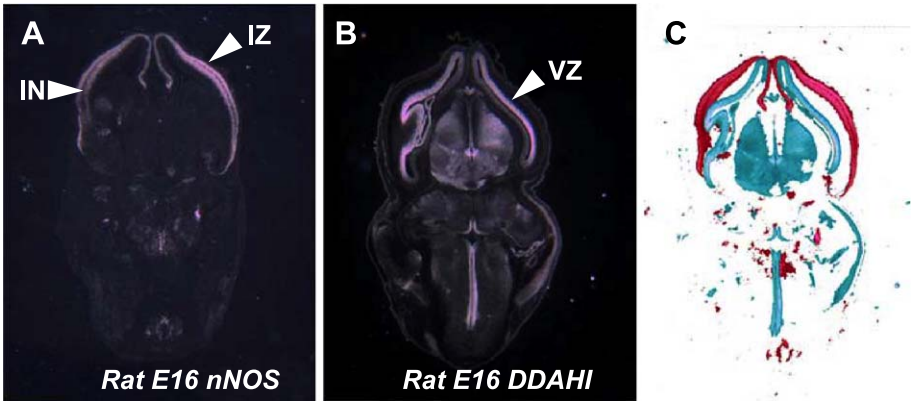

Fig. 9. In situ hybridization analyses of DDAH1 mRNA in the rat E16 brain section (A) and nNOS mRNA in an adjacent section (B). C is digitally synchronized figures A (DDAH1 signals (blue)) and B (nNOS signals (red)). IN, inner nuclear layer; IZ, intermediate zone; VZ, ventricular zone.

A *Pst*I fragment of rat nNOS cDNA (gift by Drs. Hirose and Hagiwara, Tokyo Institute of Technology) was subcloned into p-Bluescript and linealized with *Bam*HI. Radioactive cRNA antisense copies were synthesized using T7 polymerase [3].

3. Discussion

DDAH1 was first reported as an enzyme that metabolizes N^G-monomethyl-L-arginine and asymmetrical dimethylarginine to citrulline and methylamine [7] and is known to regulate nitric oxide synthase [5]. A second DDAH isoform (DDAH2) with the same function has since been isolated [4]. DDAH1 expression patterns have been detected in the central nervous system (CNS) of adult rats [6], but no developmental profile has been reported histochemically.

Tran et al. [8] has reported that human DDAH1 expression varied little between fetal and adult tissues compared to DDAH2, but organs which expressed strong DDAH1 mRNA signals in our histological results match those in their dot data results, and some difference between species is to be expected. Our histological results showed that DDAH1 mRNA signal expressions in chick and rat embryos changed radically at each stage, so it may be that not only DDAH2 but DDAH1 also plays an important role in development.

Although Leiper et al. [4] reported that the expression pattern of DDAH1 mRNA correlated with nNOS mRNA in human adult brains, our histological results indicated that, in the brains of rat embryos, the expression pattern of DDAH1 mRNA was different from that of nNOS mRNA (Fig. 9C). This may be due to the lower correlation between the DDAH1 mRNA level and DDAH1 protein level, as was reported by Arrigoni et al. [1]. Further studies will be needed to address these questions.

References

[1] F.I. Arrigoni, P. Vallance, S.G. Haworth, J.M. Leiper, Metabolism of asymmetric dimethylarginines is regulated in the lung developmentally and with pulmonary hypertension induced by hypobaric hypoxia, Circulation 107 (2003) 1195–1201.

[2] T. Hamada, K. Ui-Tei, Y. Miyata, A novel gene derived from developing spinal cords, SCDGF, is a unique member of the PDGF/VEGF family, FEBS Lett. 475 (2000) 97–102.

[3] S. Harada, T. Imaki, N. Chikada, M. Naruse, H. Demura, Distinct distribution and time-course changes in neuronal nitric oxide synthase and inducible NOS in the paraventricular nucleus following lipopolysaccharide injection, Brain Res. 821 (1999) 322–332.

[4] J.M. Leiper, J. Santa Maria, A. Chubb, R.J. MacAllister, I.G. Charles, Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases, Biochem. J. 343 (1999) 209–214.

[5] R.J. MacAllister, H. Parry, M. Kimoto, T. Ogawa, R.J. Russel, H. Hodson, G.S. Whitley, P. Vallence, Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase, Br. J. Pharmacol. 119 (1996) 1533–1540.

[6] S. Nakagomi, S. Kiryu-Seo, M. Kimoto, P.C. Emson, H. Kiyama, Dimethylarginine dimethylaminohydrolase (DDAH) as a nerve-injury-associated molecule: mRNA localization in the rat brain and its coincident up-regulation with neuronal NO synthase (nNOS) in axotomized motoneuron, Eur. J. Neurosci. 11 (1999) 2160–2166.

[7] T. Ogawa, M. Kimoto, K. Sasaoka, Purification and properties of a new enzyme, N^G,N^G-dimethylarginine dimethylaminohydrolase, from rat kidney, J. Biol. Chem. 264 (1989) 10205–10209.

[8] C.T.L. Tran, M.F. Fox, P. Vallance, J.M. Leiper, Chromosomal localization, gene structure, and expression pattern of DDAH1: comparison with DDAH2 and implications for evolutionary origins, Genomics 68 (2000) 101–105.

[9] K. Yoshida, K. Kawamura, J. Imaki, Differential expression of c-*fos* mRNA in rat retinal cells: regulation by light/dark cycle, Neuron 10 (1993) 1049–1054.